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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:

JONATHAN EDWARD LIGHTNER
JOHN JOSEPH OKULEY

CASE NO.: BB-1043-A

COPY OF PAPERS ORIGINALLY FILED

SERIAL NO.: 08/256,047

GROUP ART UNIT: 1803

FILED: OCTOBER 7,1994

EXAMINER: MCELWAIN

FOR: GENES FOR MICROSOMAL DELTA-12 FATTY ACID DESATURASES AND RELATED ENZYMES FROM PLANTS

Date: JANUARY 30, 1998

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

DECLARATION OF DR. ANTHONY JOHN KINNEY UNDER 37 CFR 1.132

I, Anthony John Kinney declare as follows:

1. I am a citizen of the United Kingdom and am a permanent resident of the United States of America, residing in Wilmington, Delaware.

2. I received a B Sc. in biology from the University of Sussex in 1980 and a D. Phil. in biochemistry and cell biology from Oxford University in 1985.

3. I served as a research fellow in the Department of Food Science at Rutgers University, New Brunswick, N.J. 9/87-5/89.

4. I have been employed at E. I. du Pont de Nemours and Company (DuPont) since June, 1989 and presently work as a principal investigator for DuPont's agricultural products and am presently working on expression of storage oil genes.

5. I have authored in excess of fifteen refereed articles in the field of biochemistry.

6. I have reviewed the above-identified case, and the Official Action for the subject case dated October 30, 1997. I understand that this declaration is being submitted to address the rejections of the pending claims under 35 USC §112 first paragraph or second paragraph. Enclosed herewith are a number of amino acid sequence alignments and phylogenetic tree depictions of numerous plant C18:1 acyl-ACP delta-12 desaturases or acyl-ACP 12-hydroxylases which were discussed with the Examiner at the interview held on December 9,

I HEREBY CERTIFY THAT THIS PAPER IS BEING DEPOSITED WITH THE UNITED STATES POSTAL SERVICE WITH SUFFICIENT POSTAGE AS FIRST CLASS MAIL IN AN ENVELOPE ADDRESSED TO: ASST. COMMISSIONER FOR PATENTS, WASHINGTON, D.C. 20231, ON THIS DATE

1/30/98
Date

1997. All of these figures were prepared using the Megalign program from DNAStar, Inc., applying the comparison method of Hein (1990, *Meth. Enz.* **183:**626-546).

The subject case discloses nucleic acid fragments encoding several plant C18:1 acyl-ACP delta-12 desaturases and a plant C18:1 12-hydroxylase which can be characterized as a delta-12 desaturase related enzyme. These nucleic acids encode proteins that form a distinct class of enzymes with regard to their amino acid sequence conservation.

Attachment I presents an alignment of the polypeptides set forth in SEQ ID NOS:2, 4, 6, 8, and 12; these are the predicted gene products from the nucleotide sequences set forth in SEQ ID NOS:1, 3, 5, 7, and 11. The shaded residues in the alignment indicate those residues that are conserved when compared with the *Arabidopsis* polypeptide (SEQ ID NO:2).

Attachment II is a phylogenetic tree depicting the calculated evolutionary/structural relationship of the sequences aligned in Attachment I. These figures clearly depict the close evolutionary/structural relationship among these proteins. Furthermore, it is also apparent that the 12-hydroxylase from castor is a member of this class of proteins. Within these alignments it can be seen that the delta-12 desaturase from soybean is more closely related to the hydroxylase from castor than it is to the other delta 12-desaturases disclosed in the subject specification. The sequences disclosed in the instant specification are also very closely conserved with other plant delta-12 desaturases.

Attachment III presents the amino acid sequence alignment of the previously mentioned polypeptides with those of other desaturases that have appeared in the public domain since the filing of the subject case. The additional sequences are for the proteins from *Brassica juncea*, parsley, potato, sunflower, and peanut (GenBank or EMBL accession numbers X91139, U86072, X92847, U91341, and AF030319, respectively). The corresponding phylogenetic tree for this expanded group of protein sequences is found (plotted on a logarithmic scale) in Attachment IV. These comparisons show clearly the relationship between these sequences, and convey graphically the expectation that any one of nucleic acid sequences set forth in SEQ ID NO 1, 3, 5, 7, or 11 may be used as a hybridization probe for genes encoding other plant delta-12 desaturases or desaturase related enzymes, such as 12-hydroxylases. This has been confirmed in my laboratory.

Delta-12 desaturase or 12-hydroxylase protein sequences are structurally distinguishable from those of the next most closely related class, the delta-15 desaturases. Attachment V presents an amino acid sequence alignment of the previously described 12-

desaturases with the sequences of plant delta-15 desaturases that were disclosed in WO 93/11245 (now pending USSN 08/244205). As suggested by the data within the specification for the subject case, it is relatively easy to identify delta 12-desaturases on the basis of their similarity, and moreover to distinguish them from the delta-15 desaturases due to their dissimilarity The shading in this figure indicates those residue that are conserved relative to the delta-12 desaturase of *Arabidopsis*. Alignment of the 12-desaturases indicates that there is approximately 50% strict conservation or identity of amino acid residues across these proteins, however, they share only 30%-35% identity with any of the delta-15 desaturases. The phylogenetic tree depiction (Attachment VI) shows quite clearly that the two classes do not overlap. Rather, their members are separated by a highly statistically significant divergence, as is indicated by the scale along the bottom of the figure. Further support for this distinction comes from failed experimental attempts to use a gene encoding a plant delta-15 desaturase as a hybridization probe to identify and isolate genes that encode plant delta-12 desaturases.

The delta-12 desaturases are members of a group of proteins that catalyze various oxidations of C18:1 fatty acyl groups at the C12-C13 bond. That is, other activities are known which catalyze mechanistically similar oxidations at this position, but do not necessarily produce a double bond in the product. However, it is not possible to say that these represent different classes of structures.

Disclosed in the subject case is the cDNA sequence from *Ricinus communis* (castor bean) encoding a 12-hydroxylase, which is a desaturase-related enzyme. The amino acid sequence of this protein is seen to fall cleanly within the "delta-12 desaturase" group when analyzed by a comparison method such as that of Hein (1990). Attachment V and VI also include within their presentations the placement of the hydroxylase from *Lesquerella fendleri* (WO 97/30582, SEQ ID NO:4). What is striking about this comparison is that the *Lesquerella* hydroxylase and the *Arabidopsis* delta-12 desaturase are more closely related to each other than they are to any other hydroxylase or desaturase included in the analysis. Not only are the proteins indistinguishable on the basis of their sequence, but it is now known that it may not be possible to distinguish them with regard to their function. Braun et al. recently described results of experiments which sought to identify amino acid residues that disposed a protein to being either a desaturase or a hydroxylase (Broun et al., in "Physiology, Biochemistry and Molecular Biology of Plant Lipids," Williams, J.P. et al., eds., Kluwer Academic Publishers, Dordrecht, 1997, pages 342-344; a copy of which is enclosed herewith). During the course of their work, the authors expressed the "hydroxylase" from *Lesquerella* in yeast under the control of an inducible promoter, and found that when

compared to the uninduced control, the induced transformant produced both the C18:2 fatty acid, linoleic acid, and the 12-hydroxy form of oleic acid, ricinoleic acid. As it is known that yeast normally does not make detectable amounts of either of these oleic acid derivatives, it follows that the single protein from *Lesquerella* is responsible for the production of both products. Accordingly, it is both a "desaturase" and a "hydroxylase." These researchers then showed that mutation of only six amino acids (out of a total of 384 residues) was sufficient to convert this to a protein with properties closer to those of a "pure" desaturase. These results are depicted graphically in Figure 1 of the reference. Thus, it is apparent that this structural and functional class of proteins certainly includes more than enzymes that catalyze desaturation.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Respectfully submitted,

AJKinney

DR. ANTHONY JOHN KINNEY

Date: 30 JAN 98

Attachment I

Alignment Report of microfad2 MEG, using J. Hein method with PAM250 residue weight table.
Monday, January 26, 1998 4:48 PM

```
    M G A G G R M X - - - - V X X S S K K - - - - - - S E T D X L K R V P C E K P P Majority
                      10                  20                  30                  40
  1 M G G R G R V A K V E V Q - - - - - - - - - - - - - G K K P L S R V P N T K P P New soyxfad2.pep
  1 M G G G G R M S T V I I S N N S E K K - - - - - - G G S S H L E R A P H T K P P CASTOR FA-OH.PEP
  1 M G A G G R M P - - - - V P T S S K K - - - - - - S E T D T T K R V P C E K P P New araxfad2.pep
  1 M G A G G R M Q - - - - V S P P S K K - - - - - - S E T D N I K R V P C E T P P New canxfad2.pep
  1 M G A G G R M T - - - - E K E R E K Q E Q L A R A T G G A A M Q R S P V E K P P cornfad2e.PRO

    F T V G Q L K K A I P P H C F E R S I L R S F S Y V V Y D L I I A S C F Y Y V A Majority
                      50                  60                  70                  80
 28 F T V G Q L K K A I P P H C F Q R S L L T S F S Y V V Y D L S F A F I F Y - I A New soyxfad2.pep
 35 Y T L G N L K F A I P P H C F E R S F V R S F S N F A Y N F C L S F L S Y S I A CASTOR FA-OH.PEP
 31 F S V G D L K K A I P P H C F K R S I P R S F S Y L I S D I I I A S C F Y Y V A New araxfad2.pep
 31 F T V G E L K K A I P P H C F K R S I P R S F S H L I W D I I I A S C F Y Y V A New canxfad2.pep
 37 F T L G Q I K K A I P P H C F E R S V L K S F S Y V V H D L V I A A A L L Y F A cornfad2e.PRO

    T T Y F P L L F S P L S Y X A W P L Y W A C Q G C V L T G V W V I A H E C G H H Majority
                      90                 100                 110                 120
 67 T T Y F H L L F Q P F S L I A W P I Y W V L Q G C L L T G V W V I A H E C G H H New soyxfad2.pep
 75 T N F F P Y I S S P L S Y V A W L V Y W L F Q G C I L T G L W V I G H E C G H H CASTOR FA-OH.PEP
 71 T N Y F S L L F Q P L S Y L A W P L Y W A C Q G C V L T G I W V I A H E C G H H New araxfad2.pep
 71 T T Y F P L L P N P L S Y F A W P L Y W A C Q G C V L T G V W V I A H E C G H A New canxfad2.pep
 77 L A I I P A L F S P L R Y A A W P L Y W I A Q G C V C T G V W V I A H E C G H H cornfad2e.PRO

    A F S D Y Q W L D D V V G L I L H S F L L V P Y F S W K Y S H R R H H S N T G S Majority
                     130                 140                 150                 160
107 A F S K Y Q W V D D V V G L T L H S T L L V P Y F S W K I S H R R H H S N T G S New soyxfad2.pep
115 A F S E Y Q L A D D I V G L I V H S A L L V P Y F S W K Y S H R R H H S N I G S CASTOR FA-OH.PEP
111 A F S D Y Q W L D D T V G L I F H S F L L V P Y F S W K Y S H R R H H S N T G S New araxfad2.pep
111 A F S D Y Q W L D D T V G L I F H S F L L V P Y F S W K Y S H R R H H S N T G S New canxfad2.pep
117 A F S D Y S L L D D V V G L V L H S S L M V P Y F S W K Y S H R R H H S N T G S cornfad2e.PRO

    L E R D E V F V P K X K S A I S W Y S K Y L - N N P L G R V V M L T V Q L T L G Majority
                     170                 180                 190                 200
147 L D R D E V F V P K P K S K V A W F S K Y L - N N P L G R A V S L L V T L T I G New soyxfad2.pep
155 L E K D E V F V P K S K S K I S W Y S K Y L - N N P P G R V L T L A A T L L L G CASTOR FA-OH.PEP
151 L E R D E V F V P K Q K S A I K W Y G K Y L - N N P L G R I M M L T V Q F V L G New araxfad2.pep
151 L E R D E V F V P R R S Q T S S - G T A S T - S T T F G R T V M L T V Q F T L G New canxfad2.pep
157 L E R D E V F V P K K K E A L P W Y T P Y V Y N N P V G R V V H I V V Q L T L G cornfad2e.PRO

    W P L Y L A F N V S G R P Y D G - F A C H F D P Y A P I Y N D R E R L Q I Y V S Majority
                     210                 220                 230                 240
186 W P M Y L A F N V S G R P Y D S - F A S H Y H P Y A P I Y S N R E R L L I Y V S New soyxfad2.pep
194 W P L Y L A F N V S G R P Y D [illegible] - F A C H [illegible] P I F [illegible] R F E R L Q I Y I A CASTOR FA-OH.PEP
190 W P L Y L A F N V S G R P Y D G - F A C H F F P N A P I Y N D R E R L Q I Y L S New araxfad2.pep
189 W P L Y L A F N V S G R P Y D G - F A C H F [illegible] P N A P I Y N D R E R L Q I Y [illegible] New canxfad2.pep
197 W P L Y L A [illegible] N - S G R P Y P P - F A C H F [illegible] P [illegible] P I Y N D R E R A Q I F V S cornfad2e.PRO
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Alignment Report of microfad2 MEG, using J. Hein method with PAM250 residue weight table.
Monday, January 26, 1998 4:48 PM

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       D A G I L A V T F G L Y R Y A A A Q G V A W V V C V Y G V P L L I V N G F L V L  Majority
                          250                 260                 270                 280
   25  D V A L F S V T Y S L Y R V A T L K G L V W L L C V Y G V P L L I V N G F L V T  New soyxfad2.pep
   33  D L G I F A T T F V L Y Q A T M A K G L A W V M R I Y G V P L L I V N C F L V M  CASTOR FA-OH.PEP
   29  D A G I L A V C F G L Y R Y A A A Q G M A S M I C L Y G V P L L I V N A F L V L  New araxfad2.pep
   29  D A G I L A V C Y G L L F Y A A V Q G V A S M V C F L R V P L L I V N G F L V L  New canxfad2.pep
   36  D A G V V A V A F G L Y F L A A A F G V W W V V R V Y A V P L L I V N A W L V L  cornfad2e.PRO

       I T Y L Q H T H P S L P H Y D S S E W D W L R G A L A T V D R D Y G I L N K V F  Majority
                          290                 300                 310                 320
   65  I T Y L Q H T H F A L P H Y D S S E W D W L K G A L A T M D R D Y G I L N K V F  New soyxfad2.pep
   73  I T Y L Q H T H P A I P F Y G S S E W D W L R G A M V T V D R D Y G V L N K V F  CASTOR FA-OH.PEP
   69  I T Y L Q H T H P S L P H Y D S S E W D W L R G A L A T V D R D Y G I L N K V F  New araxfad2.pep
   69  I T Y L Q H T H P S L P H Y D S S E W D W L R G A L A T V D R D Y G I L N Q G F  New canxfad2.pep
   76  I T Y L Q H T H P S L P H Y D S S E W D W L R G A L A T M D R D Y G I L N R V F  cornfad2e.PRO

       H N I T D T H V A H H L F S T M P H Y H A M E A T K A I K P I L G E Y Y Q F D G  Majority
                          330                 340                 350                 360
  305  H H I T D T H V A H H L F S T M P H Y H A M E A T N A I K P I L G E Y Y Q F D D  New soyxfad2.pep
  313  H N I A D T H V A H H L F A T V P H Y H A M E A T K A I K P I M G E Y Y R Y D G  CASTOR FA-OH.PEP
  309  H N I T D T H V A H H L F S T M P H Y N A M E A T K A I K P I L G D Y Y Q F D G  New araxfad2.pep
   09  H N I T D T H E A H H L F S T M P H Y H A M E A T K A I K P I L G E Y Y Q F D G  New canxfad2.pep
   16  H N I T D T H V A H H L F S T M P H Y H A M E A T K A I R P I L G D Y Y H F D P  cornfad2e.PRO

       T P V Y K A L W R E A K E C I Y V E P D E G G D K K G V Y W Y N N K Y            Majority
                          370                 380                 390
[illegible]  T P F Y K A L W R E A R E C L Y V E P D E G T S E K G V Y W Y R N K Y            New soyxfad2.pep
   53  T P F Y K A L W R E A K E C L F V E P D E G A P T Q G V F W Y R N K Y            CASTOR FA-OH.PEP
   49  T P W Y V A M Y R E A K E C I Y V E P D R E G D K K G V Y W Y N N K L            New araxfad2.pep
   49  T P V V K A M W R E A K E C I Y V E P D R Q G E K K G V F W Y N N                New canxfad2.pep
   56  T P V A K A T W R E A G E C I Y V E R E - - - D R K G V F W Y N K K F            cornfad2e.PRO

Decoration 'Decoration #2': Shade (with bright turquoise at 40% fill) residues that match
New araxfad2.pep exactly.
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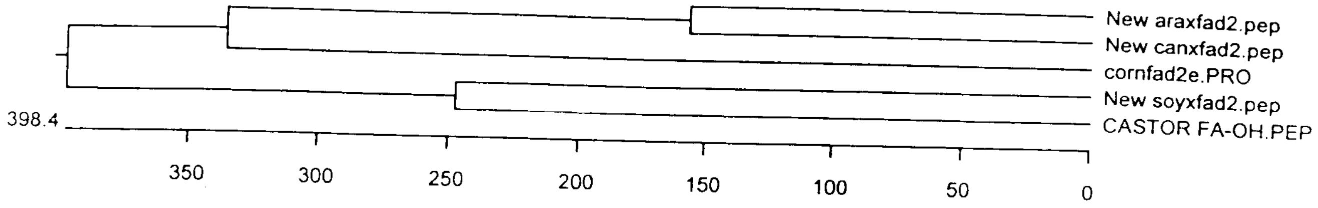
New soyxfad2.pep = soybean delta-12 desaturase
CASTOR FA-OH.PEP = castor 12-hydroxylase
New araxfad2.pep = Arabidopsis delta-12 desaturase
New canxfad2.pep = canola delta-12 desaturase
cornfad2e.PRO = corn delta-12 desaturase

Attachment II

Phylogenetic tree of microfad2.MEG, using J in method with PAM250 residue weight table.
Monday, January 26, 1998 4:47 PM



New soyxfad.2pep = soybean delta-12 desaturase
CASTOR FA-OH.PEP = castor 12-hydroxylase
New araxfad2.pep = Arabidopsis delat-12 desaturase
New canxfad2.pep = canola delta-12 desaturase
cornfad2e.PRO = corn delta-12 desaturase

Attachment III

nt Report of Uspto2.MEG, using J. Hein method with PAM250 residue weight table.
January 26, 1998 5:24 PM

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G A G G R M - - - - S S - - - - K K S E - K - - - - - - - - - - - N P - - - - -  Majority
                  10                  20                  30                  40

G G R G R V - - - - A K - - - - V E V Q G K - - - - - - - - - - - K P - - - - -  New soyxfad2.pep
G G G G R M - - - - S T - - - - V I I S - N - - - - - - - - - - - N S - - - E K  CASTOR FA-OH PEP
G A G G R M P V P T S S - - - - K K S E - T - - - - - - - - - - - D T - - - - -  New araxfad2 pep
G A G G R M Q V S P P S - - - - K K S E - T - - - - - - - - - - - D N - - - - -  New canxfad2.pep
G A G G R M - - - - T E - - - - K E R E - K Q E Q L A R A T G G A A - - - - - -  cornfad2e.PRO
G A G G R M Q V S P S P - - - - K K S E - T - - - - - - - - - - - D T - - - - -  bjunfad2.PRO
G A G E - Y - - - - T S - - - - V T N E - N - - - - - - - - - - - N P - - - - -  helianfad2.PRO
G A G G R M - - - - P I - - - - N P Q G - K - - - - - - - - - - - N S E E V - -  petroselFad2.PRO
G A G G R M - - - - S A P N G E T E V K - R - - - - - - - - - - - N P - - - - -  potatoFad2.PRO
G A G G R V - - - - T K - - - - I E A Q - K - - - - - - - - - - - K P - - - - -  Peanut.PRO

- - - - - L Q R V P H E K P P F T V G D L K K A I P P H C F K R S L P R S F S  Majority
                50                  60                  70                  80

- - - - - L S R V P N T K P P F T V G Q L K K A I P P H C F Q R S L L T S F S  New soyxfad2.pep
G G S S H L E R A P H T K P P Y T L G N L K R A I P P H C F E R S F V R S F S  CASTOR FA-OH.PEP
  - - - - T K R V P C E K P P F S V G D L K K A I P P H C F K R S I P R S F S  New araxfad2.pep
- - - - - I K R V P C E T P P F T V G E L K K A I P P H C F K R S I P R S F S  New canxfad2.pep
- - - - - M Q R S P V E K P P F T L G Q I K K A I P P H C F E R S V L K S F S  cornfad2e.PRO
- - - - - L K R V P C E T P P F T V G E L K K A I P P H C F K R S I P R S F S  bjunfad2.PRO
- - - - - L D R V P H A K P P F T I G D L K K A I P P H C F Q R S L T R S F S  helianfad2.PRO
- - - - - L Q R V P V S K P P F T V G D I K K A I P P H C F K R S V I R S F S  petroselFad2.PRO
- - - - - L Q K V P T S K P P F T V G D I K K A I P P H C F Q R S L I R S F S  potatoFad2.PRO
- - - - - L S R V P H S N P P F S V G Q L K K A I P P H C F E R S L F I S F S  Peanut.PRC

V V Y D L I I A S C L Y Y V A T T Y F H L L P S P L S Y I A W P L Y W A C Q G  Majority
                90                 100                 110                 120

V V Y D L S F A F - I F Y I A T T Y F H L L P Q P F S L I A W P I Y W V L Q G  New soyxfad2.pep
F A Y N F C L S F L S Y S I A T N F F F Y I S S P L S Y V A W L V Y W L F Q G  CASTOR FA-OH.PEP
L I S D I I I A S C F Y Y V A T N Y F S L L P Q P L S Y L A W P L Y W A C Q G  New araxfad2.pep
L I W D I I I A S C F Y Y V A T T Y F F L L P N P L S Y F A W P L Y W A C Q G  New canxfad2.pep
V V H D L V I A A A L L Y F A L A I I F A L P S P L R Y A A W P L Y W I A Q G  cornfad2e.PFO
L I W D I I V A S C F Y Y V A T T Y F F L L P H P L S Y V A W P L Y W A C Q G  bjunfad2.PRO
V L S D L T I T A V L Y H I A T T Y F H H L P T P L S S I A W A S Y W V V Q G  helianfad2.PRO
V V Y D L V I A S I L Y F V A T N Y I Q L L P H P L S Y I A W P I H G F V Q G  petroselFad2.PRO
V V Y D L I L V S I M Y Y V A N T Y F H L L P S P Y C Y I A W P I Y W I C Q G  potatoFad2.PRO
V V Y D L L V A Y L L F Y I A T T Y F H K L P Y P F S F L A W P I Y W A I Q G  Peanut.PRO

V L T G V W V I A H E C G H H A F S D Y Q W L D D T V G L I L H S S L L V P Y  Majority
               130                 140                 150                 160

V L T G V W V I A H E C G H H A F S K Y Q W V D D T V G L T I H S T L L V P Y  New soyxfad2.pep
I L T G L W V I G H E C G H H A F S E Y Q L A D D I V G L I V H S A L L V P Y  CASTOR FA-OH.PEP
V L T G I W V I A H E C G H H A F S D Y Q W L D D T V G L I F H S F L L V P Y  New araxfad2.pep
V L T G V W V I A H E C G H A A F S D Y Q W L D D T V G L I F H S F L L V P Y  New canxfad2.pep
V L T G V W V I A H E C G H H A F S D Y - - L D D V V G L V I H S S L M V P Y  cornfad2e.PRO
V L T G V W V I A H D C G H H A F S D Y Q W L D D T V G L I F H S F L L V P Y  bjunfad2.PRO
V L T G V W V I A H E C G H H A F S D Y Q W V D D T V G F V L H S F L L V P Y  helianfad2.PRO
V L T G I W V I A H E C G H H A F S D Y Q W L D D T V G L I I H S S L L V P Y  petroselFad2.PRO
V L T G I W V N A H E C G H H A F S D Y Q W V D D T V G L I L H S A L L V P Y  potatoFad2.PRO
I L T G V W V I A H E C G H H A F S K Y Q L V D D M V G L I I H S A L L V P Y  Peanut.PRO
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S W K Y S H R R H H S N T G S L E R D E V F V P K P K S K V S W Y S K Y L - N  Majority

                   170                 180                 190                 200

S W K I S H R R H H S N T G S L D R D E V F V P K P K S K V A W F S K Y L - N  New soyxfad2.pep
S W K Y S H R R H H S N I G S L E R D E V F V P K S K S K I S W Y S K Y L - N  CASTOR FA-OH.PEP
S W K Y S H R R H H S N T G S L E R D E V F V P K Q K S A I K W Y G K Y L - N  New araxfad2.pep
S W K Y S H R R H H S N T G S L E R D E V F V P R R S Q T S S - G T A S T - S  New canxfad2.pep
S W K Y S H R R H H S N T G S L E R D E V F V P K K K E A L P W Y T P Y V Y N  cornfad2e.PRO
S W K Y S H R R H H S N T G S L E R D E V F V P K K K S D I K W Y G K Y L - N  bjunfad2.PRO
S W K Y S H H R H H S N T G S L E R D E V F V P K S R S K V P W Y S K Y F - N  helianfad2.PRO
S W K Y S H R R H H S N T G S L E R D E V F V P K P K S E L Q S V A K Y T - N  petroselFad2.PRO
S W K Y S H R R H H S N T G S L E R D E V F V P K P K S Q L G W Y S K Y L - N  potatoFad2.PRO
S W K I S H R R H H S N T G S L D R N E V F V P K P K S K V S W Y N K Y M - N  Peanut.PRO

P P G R V V S L T V T L T L G W P L Y L A F N V S G R P Y D R - F A C H Y D P  Majority

                   210                 220                 230                 240

P L G R A V S L L V T L T I G W P M Y L A F N V S G R P Y D S - F A S H Y H P  New soyxfad2.pep
P P G R V L T L A A T L L L G W P L Y L A F N V S G R P Y D R - F A C H Y D P  CASTOR FA-OH.PEP
P L G R I M M L T V Q F V L G W P L Y L A F N V S G R P Y D G - F A C H F E P  New araxfad2.pep
T F G R T V M L T V Q F T L G W P L Y L A F N V S G R P Y D G G F A C H F H P  New canxfad2.pep
P V G R V V H I V V Q L T L G W P L Y L A T N A S G R P Y P R - F A C H F D P  cornfad2e.PRO
P L G R T V M L T V Q F T L G W P L Y W A F N V S G R P Y P E G F A C H F H P  bjunfad2.PRO
T V G R I V S M F V T L T L G W P L Y L A F N V S G R P Y D R - F A C H Y V P  helianfad2.PRO
P P G R V L T L L V T L T L G W P L Y L L F N V S G R H Y E R - F A C H Y D P  petroselFad2.PRO
P P G R V L S L T I T L T L G W P L Y L A F N V S G R P Y D R - F A C H Y D P  potatoFad2.PRO
P P G R A I S L F I T L T L G W P L Y L A F N V S G R P Y D R - F A S H Y D P  Peanut.PRO

A P I Y N D R E R L Q I Y V S D A G I L A V C Y G L Y R Y A A A K G L A W V V  Majority

                   250                 260                 270                 280

A P I Y S N R E R L L I Y V S D V A L F S V T Y S L Y R V A T L K G L V W L L  New soyxfad2.pep
G P I F S E R E R L Q I Y I A D L G I F A T T F V L Y Q A T M A K G L A W V M  CASTOR FA-OH.PEP
A P I Y N D R E R L Q I Y L S D A G I L A V C F G L Y R Y A A A Q G M A S M I  New araxfad2.pep
A P I Y N D R E R L Q I Y I S D A G I L A V C Y G L L P Y A A V Q G V A S M V  New canxfad2.pep
G P I Y N D R E R A Q I F V S D A G V V A V A F G L Y K L A A A F G V W W V V  cornfad2e.PRO
A P I Y N D R E R L Q I Y V S D A G I L A V C Y G L Y R Y A A A Q G V A S M V  bjunfad2.PRO
S P M Y N E R K R Y Q I V M S D I G I V I T S F I L Y R V A M A K G L V W V I  helianfad2.PRO
S P I Y S D R E R A Q I L V S D A G V L A V S Y G L Y R L A V A K G L T W V L  petroselFad2.PRO
G P I Y N N R E R L Q I F I S D A G V L G V C Y L L Y R I A L V K G L A W L V  potatoFad2.PRO
A P I Y S N R E R L L I Y V S D S S V F A V T Y L L Y H I A T L K G L G W V V  Peanut.PRO

L Y G V P L L I V N G F L V L I T Y L Q H T H P S L P H Y D S S E W D W L R G  Majority

                   290                 300                 310                 320

L Y G V P L L I V N G F L V T I T Y L Q H T H P A L P H Y D S S E W D W L R G  New soyxfad2.pep
L Y G V P L L I V N C F L V M I T Y L Q H T H P A I P R Y G S S E W D W L R G  CASTOR FA-OH.PEP
L Y G V P L L I V N A F L V L I T Y L Q H T H P S L P H Y D S S E W D W L R G  New araxfad2.pep
L Y G V P L L I V N A F L V L I T Y L Q H T H P S L P H Y D S S E W D W L R G  New canxfad2.pep
L Y A V P L L I V N A W L V L I T Y L Q H T H P S L P H Y D S S E W D W L R G  cornfad2e.PRO
L Y G V P L L I V N A F L V L I T Y L Q H T H P S L P H Y D S S E W D W L R G  bjunfad2.PRO
L Y G V P L M V V N A F L V L I T Y L Q H T H P G L P H Y D S S E W E W L K G  helianfad2.PRO
L Y G V P L L I V N G F L V L I T F L Q H T H P A L P H Y N S S E W D W L R G  petroselFad2.PRO
L Y G V P L L V V N G F L V L I T Y L Q H T H P S L P H Y D S T E W D W L R G  potatoFad2.PRO
L Y G V P L L I V N G F L V T I T Y L Q H T H A S L P H Y D S S E W D W L R G  Peanut.PRO
```

ent Report of Uspto2 MEG, using J. Hein method with PAM250 residue weight table.
January 26, 1998 5:24 PM

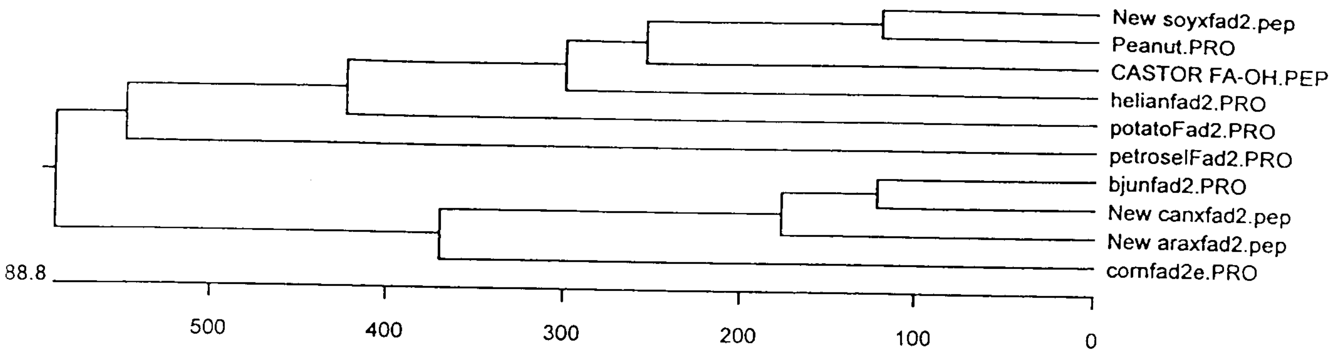
```
L A T V D R D Y G I L N K V F H N I T D T H V A H H L F S T M P H Y H A M E A  Majority

                 330                 340                 350                 360
L A T M D R D Y G I L N K V F H H I T D T H V A H H L F S T M P H Y H A M E A  New soyxfad2.pep
M V T V D R D Y G V L N K V F H N I A D T H V A H H L F A T V P H Y H A M E A  CASTOR FA-OH.PEP
L A T V D R D Y G I L N K V F H N I T D T H V A H H L F S T M P H Y N A M E A  New araxfad2.pep
L A T V D R D Y G I L N Q G F H N I T D T H E A H H L F S T M P H Y H A M E A  New canxfad2.pep
L A T M D R D Y G I L N R V F H N I T D T H V A H H L F S T M P H Y H A M E A  cornfad2e.PRO
L A T V D R D Y G I L N K V F H N I T D T H V A H H L F S T M P H Y H A M E V  bjunfad2.PRO
L A T V D R D Y G V L N K V F H H I T D T H V V H H L F S T M P H Y N A M E A  helianfad2.PRO
L A T V D R D Y G I L N K V L H N I T D T H V A H H L F S T M P H Y N A M E A  petroselFad2.PRO
L A T C D R D Y G V L N K V F H N I T D T H V V H H L F S T M P H Y N A M E A  potatoFad2.PRO
L A T V D R D Y G I L N K A F H H I T D T H V A H H L F S T M P H Y H A M E A  Peanut.PRO

K A I K P I L G D Y Y Q F D G T P F Y K A M W R E A K E C L Y V E P D E G G S  Majority

                 370                 380                 390                 400
N A I K P I L G E Y Y Q F D D T P F Y K A L W R E A R E C L Y V E P D E G T S  New soyxfad2.pep
K A I K P I M G E Y Y R Y D G T P F Y K A L W R E A K E C L F V E P D E G A P  CASTOR FA-OH.PEP
K A I K P I L G D Y Y Q F D G T P W Y V A M Y R E A K E C I Y V E P D R E G D  New araxfad2.pep
K A I K P I L G E Y Y Q F D G T P V V K A M W R E A K E C I Y V E P D R Q G E  New canxfad2.pep
K A I R P I L G D Y Y H F D P T P V A K A T W R E A G E C I Y V E P E D - - -  cornfad2e.PRO
K A I K P I L G D Y Y Q F D G T P W V K A M W R E A K E C I Y V E P D R Q G E  bjunfad2.PRO
K A L R P V L G E Y Y R F D K T P F Y V A M W R E M K E C L F V E Q D D E G K  helianfad2.PRO
K A I K P I L G D Y Y R F D D T P V V K A M W R E A K E C L Y V E P D E G D Q  petroselFad2.PRO
K A V K P L L G D Y Y Q F D G T P I Y K E M W R E A K E C L Y V E K D E S S Q  potatoFad2.PRO
N A I K P I L G D Y Y Q F D G T P F Y K A L W R E A K E C L Y V E P D D G A S  Peanut.PRO

K G V F W Y N N K L - - - - -                                                  Majority

                 410
K G V Y W Y K N K Y                                                            New soyxfad2.pep
Q G V F W Y K N K Y                                                            CASTOR FA-OH.PEP
K G V Y W Y N N K L                                                            New araxfad2.pep
K G V F W Y N N                                                                New canxfad2.pep
K G V F W Y N K K F                                                            cornfad2e.PRO
K G V F W Y N N K L                                                            bjunfad2.PRO
- G V F W Y K N K M - - N                                                      helianfad2.PRO
K G I F W Y N N K L - - - -                                                    petroselFad2.PRO
K G V F W Y K N K L -                                                          potatoFad2.PRO
K G V Y W Y K N K F                                                            Peanut.PRO
```

tion Decoration #2 : Shade (with bright turquoise at 40% fill) residues that match
araxfad2.pep exactly.

New soyxfad2.pep = soybean delta-12 desaturase
CASTOR FA-OH.PEP = castor 12-hydroxylase
New araxfad2.pep = Arabidopsis delta -12 desaturase
New canxfad2.pep = canola delta-12 desaturase
cornfad2e.PRO = corn delta-12 desaturase
bjunfad2.PRO = Brassica juncea delta-12 desaturase
helianfad2.PRO = sunflower delta-12 desaturase
petroselFad2.PRO = parsely delta-12 desaturase
potatoFad2.PRO = potato delta-12 desaturase

Attachment IV

ylogenetic tree of Uspto2.MEG, using J. Hein method with PAM250 residue weight table.
nday, January 26, 1998 5:23 PM



New soyxfad2.pep = soybean delta-12 desaturase
CASTOR FA-OH.PEP = castor 12-hydroxylase
New araxfad2.pep = *Arabidopsis* delta-12 desaturase
New canxfad2.pep = canola delta-12 desaturase
cornfad2e.PRO = corn delta-12 desaturase
bjunfad2.PRO = *Brassica juncea* delta-12 desaturase
helianfad2.PRO = sunflower delta-12 desaturase
petroselFad2.PRO = parsley delta-12 desaturase
potatoFad2.PRO = potato delta-12 desaturase

Attachment V

```
M - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - Majority
                  10                  20                  30                  40
M - - - - - - - - - - - - - - - - - - - - - - - - - - - - - V - - - - - - - - - New araxfad3.pep
M A N L V L S E C G I R P L P R I Y T T P R S N F L S N N N K - - F R P S - - - New araxfadd.pep
M - - - - - - - - - - - - - - - - - - - - - - - - - - - - - V - - - - - - - - - New canxfad3.pep
F - - - - - - - - - - - - - - - - - - - - - - - - - - - - - K - - F R Q S - - - New canxfadd.pep
M - - - - - - - - - - - - - - - - - - - - - - - - - - - - - V - - - - - - - - - New soyxfad3.pep
M - - - - - - - - - - - - - - - - - - - - - - - - - - - - - A T W Y H Q K C G L New soyxfadd.pep
M - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - New soyxfad2.pep
M - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - CASTOR FA-OH.PEP
A - - - - - - - - - - - - - - - - - - - - - - - - - - - - - S - - - - - - - - - CORN FAD2.PEP
M - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - New araxfad2.pep
M - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - New canxfad2.pep
M - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - lesqOH.pro
M - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - bjunfad2.PRO
M - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - helianfad2.PRO
M - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - petroselFad2.PRO
M - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - potatoFad2.PRO

- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - Majority
                  50                  60                  70                  80
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - New araxfad3.pep
  - - - - - - - - - - - - - - - - - - - - - - - - - - - - - L S S S S Y - - - - New araxfadd.pep
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - New canxfad3.pep
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - P S S P R F - - - - New canxfadd.pep
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - New soyxfad3.pep
K P L A P V I P R P R T G A A L S S T S R V E F L D T N K V V A G P K F Q P L R New soyxfadd.pep
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - New soyxfad2.pep
                              - - - - - - - - - - - - - - - - - - - - - - - - - CASTOR FA-OH.PEP
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - CORN FAD2.PEP
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - New araxfad2.pep
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - New canxfad2.pep
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - lesqOH.pro
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - bjunfad2.PRO
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - helianfad2.PRO
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - petroselFad2.PRO
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - potatoFad2.PRO
```

New soyxfad2.pep = soybean delta-12 desaturase
CASTOR FA-OH.PEP = castor 12-hydroxylase
New araxfad2.pep = Arabidopsis delta-12 desaturase
New canxfad2.pep = canola delta-12 desaturase
CORN FAD2.PEP = corn delta-12 desaturase
bjunfad2.PRO = Brassica juncea delta-12 desaturase
helianfad2.PRO = sunflower delta-12 desaturase
petroselFad2.PRO = parsley delta-12 desaturase
potatoFad2.PRO = potato delta-12 desaturase
lesqOH = Lesquerella fendleri 12-hydroxylase
New araxfadd.pep = Arabidopsis plastidic delta-15 desaturase
New canxfadd.pep = canola plastidic delta-15 desaturase
New soyxfadd = soybean plastidic delta-15 desaturase
New soyxfad3 = soybean microsomal delta-15 desaturase
New araxfad3.pep = Arabidopsis microsomal delta-15 desaturase
New canxfad3.pep = canola microsomal delta-15 desaturase

```
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  Majority

                90                      100                       110                       120

- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  New araxfad3.pep
K T S S S P L S F G L N S R D G F T R N W A L - - - - - - - - - - - - - - - -  New araxfadd.pep
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  New canxfad3.pep
R L N - - - - - - - - - - - - - - S R N W A L - - - - - - - - - - - - - - - -  New canxfadd.pep
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  New soyxfad3.pep
N L R - - - - - - - - - - - - - - E R N W G L - - - - - - - - - - - - - - - -  New soyxfadd.pep
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  New soyxfad2.pep
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  CASTOR FA-OH.PEP
- - - - - - - - - - - - - - - - - - - - - - - P L P P P C K S C R H H R S F F  CORN FAD2.PEP
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  New araxfad2.pep
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  New canxfad2.pep
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  lesqOH.pro
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  bjunfad2.PRO
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  helianfad2.PRO
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  petroselFad2.PRO
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  potatoFad2.PRO

- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - G A G  Majority

              130                       140                       150                       160

- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  New araxfad3.pep
- - - - - - - - - - - - - - - - - - - - - - - - N V S T P L T T - - - - - - -  New araxfadd.pep
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -  New canxfad3.pep
- - - - - - - - - - - - - - - - - - - - - - - - N V T T P L T V - - - - - - -  New canxfadd.pep
- - - - - - - - - - - - - - - - - - - - - - - - K D T K P L A Y - - - - - - -  New soyxfad3.pep
- - - - - - - - - - - - - - - - - - - - - - - - K V S A P L R V - - - - - - -  New soyxfadd.pep
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - G G R  New soyxfad2.pep
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - G G G  CASTOR FA-OH.PEP
P G Q E K R G E R G E A R C P P D L L C P D A A V T T S S V S V R S K M G A G  CORN FAD2.PEP
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - G A G  New araxfad2.pep
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - G A G  New canxfad2.pep
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - G A G  lesqOH.pro
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - G A G  bjunfad2.PRO
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - G A G  helianfad2.PRO
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - G A G  petroselFad2.PRO
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - G A G  potatoFad2.PRO
```

```
G R M A V S P S S K - - - - - - - - - - K S - - - - - - - E - - - - - - - - - T  Majority
                  |                   |                   |                   |
                 170                 180                 190                 200
- - V A M D Q R T N - - - - - - - - - - V N - - - - - - - G D P G A G D R K K E  New araxfad3.pep
- - P I F E E S P L - - - - - - - - - - E E - - - - - - - D - - - - - - - - - N  New araxfadd.pep
  - V A M D Q R S N - - - - - - - - - - A N - - - - - - - G - - - - - - - - - D  New canxfad3.pep
  - D S S S S P P I - - - - - - - - - - E E - - - - - - - E - - - - - - - - - P  New canxfadd.pep
  - A A N N G Y Q Q -       - - - - - - K G - - - - - - - S - - - - - - - - - S  New soyxfad3.pep
- - A S I E E E Q K S V D L - - - - - - T N - - - - - - - G - - - - - - - - - T  New soyxfadd.pep
G R V A K V - - - E - - - - - - - - - - V Q - - - - - - - G - - - - - - - - - K  New soyxfad2.pep
G R M S T V - - - I - - - - - - - - - - I S N N S E K K G G - - - - - - - - - S  CASTOR FA-OH.PEP
G R M T E K E R E K - - - - Q E Q L A R A T - - - - - - - G - - - - - - - - - G  CORN FAD2.PEP
G R M P V P T S S K - - - - - - - - - - K S - - - - - - - E - - - - - - - - - T  New araxfad2.pep
G R M Q V S P P S K - - - - - - - - - - K S - - - - - - - E - - - - - - - - - T  New canxfad2.pep
G R I M V T P S S K - - - - - - - - - - K S - - - - - - - E - - - - - - - - - T  lesqOH.pro
G R M Q V S P S P K - - - - - - - - - - K S - - - - - - - E - - - - - - - - - T  bjunfad2.PRO
E - - Y T S - - - V - - - - - - - - - - T N - - - - - - - E - - - - - - - - - N  helianfad2.PRO
G R M P I N P Q G K - - - - - - - - - - N S - - - - - - - E - - - - - - - - - -  petroselFad2.PRO
G R M S A P N G E T - - - - - - - - - - E V - - - - - - - K - - - - - - - - - R  potatoFad2.PRO

E - - - - - - - - - P - L D R V P C A K P P F T V G D I K K A I P P H C F V R S  Majority
                  |                   |                   |                   |
                 210                 220                 230                 240
E - - - - - - - - - R - F D - - P S A Q P P F K I G D I R A A I P K H C W V K S  New araxfad3.pep
K - - - - - - - - - Q R F D - - P G A P P P F N L A D I F A A I P K H C W V K N  New araxfadd.pep
E - - - - - - - - - R - F D - - P S A Q P P F K I G D I P A A I P K H C W V K S  New canxfad3.pep
K T - - - - - - - - Q R F D - - P G A P P P F N L A D I R A A I P K H C W V K N  New canxfadd.pep
F - - - - - - - - - D - F D - - P S A P P P F K I A E I P A S I P K H C W V K N  New soyxfad3.pep
N - G V E H E K L P E - F D - - P G A P P P F N L A D I R A A I P K H C W V K D  New soyxfadd.pep
- - - - -           P   L S R V P N T K P P F T V G Q L K K A I P P H C F Q R S  New soyxfad2.pep
                    H   L K R A P H T K P P Y T L G N L K R A I P P H C F E R S  CASTOR FA-OH.PEP
A - - - - - - - - - A - M Q R S P V E K P P F T L G Q I K K A I P P H C F E R S  CORN FAD2.PEP
D - - - - - - - - - T - T K R V P C E K P P F S V G D L K K A I P P H C F K R S  New araxfad2.pep
D - - - - - - - - - N - I K R V P C E T P P F T V G E L K K A I P P H C F K R S  New canxfad2.pep
E - - - - - - - - - A - L K R G P C E K P P F T V L D L K K A I P Q H C F K R S  lesqOH.pro
D - - - - - - - - - T - L K R V P C E T P P F T V G E L K K A I P P H C F K R S  bjunfad2.PRO
N - - - - - - - - - P - L D R V P H A K P P F T I G D L K K A I P P H C F Q R S  helianfad2.PRO
E - - - - - - - - - V - L Q R V P V S K P P F T V G D I K K A I P P H C F K R S  petroselFad2.PRO
N - - - - - - - - - P - L Q K V P T S K P P F T V G D I K K A I P P H C F Q R S  potatoFad2.PRO
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F L R S F S Y V V R D L I I V S A L Y - V A T T Y F P L - - L P - P L S - I - A Majority
                    250                 260                 270                 280

P L R S M S Y V V R D L L A V A A L A - I A A V Y V D S W F L - - - - - - - - - New araxfad3.pep
P W K S L S Y V V R D V A I V F A L A - A G A A Y L N N W I V - - - - - - - - - New araxfadd.pep
P L R S M S Y V A R D Y F A V V A L A - V A A V Y F D S W F F - - - - - - - - - New canxfad3.pep
P W K S M S Y V V R E L A I V F A L A - A G A A Y L N N W L V - - - - - - - - - New canxfadd.pep
P W R S L S Y V L R D V L V I A A L V - A A A I H F D N W L L - - - - - - - - - New soyxfad3.pep
P W R S M S Y V V R D V I A V F G L A - A A A A Y L N N W L V - - - - - - - - - New soyxfadd.pep
L L T S F S Y V V Y D L S F A F I F Y - I A T T Y F H L - - L P Q P F S - L I A New soyxfad2.pep
F V R S F S N F A Y N F C L S F L S Y S I A T N F F P Y - - I S S P L S - Y V A CASTOR FA-OH.PEP
V L K S F S Y V V H D L V T A A A L L Y F A L A I I P A - - L P S P L R - Y A A CORN FAD2.PEP
I P R S F S Y L I S D I I I A S C F Y Y V A T N Y F S L - - L P Q P L S - Y L A New araxfad2.pep
I P R S F S H L I W D I I I A S C F Y Y V A T T Y F P L - - L P N P L S - Y F A New canxfad2.pep
I P R S F S Y I L T D L T L V S C F Y Y V A T N Y F S L - - L P Q P L S T Y L A lesqOH.pro
I P R S F S Y L I W D I I V A S C F Y Y V A T T Y F P L - - L P H P L S - Y V A bjunfad2.PRO
L T R S F S Y V L S D L T I T A V L Y H I A T T Y F H H - - L P T P L S - S I A helianfad2.PRO
V I R S F S Y V V Y D L V I A S I L Y F V A T N Y I Q L - - L P H P L S - Y I A petroselFad2.PRO
L I R S F S Y V V Y D L I L V S I M Y Y V A N T Y F H L - - L P S P Y C - Y I A potatoFad2.PRO

W P L Y W A A Q G C V L T G I W V I G H E C G H H A F S D Y Q W L D D T V G L I Majority
                  290                 300                 310                 320

W P L Y W A A Q G T L F W A I F V L G H D C G H G S F S D I P L L N S V V G H I New araxfad3.pep
W P L Y W L A Q G T M F W A L F V L G H D C G H G S F S N D P K L N S V V G H L New araxfadd.pep
W P L Y W A A Q G T L F W A L F V L G H D C G H G S F S D I P L L N T A V G H I New canxfad3.pep
W P L Y W I A Q G T M F W A L F V L G H D C G H G S F S N D P R L N S V V G H L New canxfadd.pep
W L I Y C P I Q G T M F W A L F V L G H D C G H G S F S D S P L L N S L V G H I New soyxfad3.pep
W P L Y W A A Q G T M F W A L F V L G H D C G H G S F S N N S K L N S V V G H L New soyxfadd.pep
W P I Y W V L Q G C L L T G V W V I A H E C G H H A F S K Y Q W V D D V V G L T New soyxfad2.pep
W L V Y W L F Q G C I L T G L W V I G H E C G H H A F S E Y Q L A D D I V G L I CASTOR FA-OH.PEP
W P L Y W I A Q G C V C T G V W V I A H E C G H H A F S D Y S L W D D V V G L V CORN FAD2.PEP
W P L Y W A C Q G C V L T G I W V I A H E C G H H A F S D Y Q W L D D T V G L I New araxfad2.pep
W P L Y W A C Q G C V L T G V W V I A H E C G H A A F S D Y Q W L D D T V G L I New canxfad2.pep
W P L Y W V C Q G C V L T G I W V L G H E C G H H A F S D Y Q W V D D T V G F I lesqOH.pro
W P L Y W A C Q G V V L T G V W V I A H E C G H H A F S D Y Q W L D D T V G L I bjunfad2.PRO
W A S Y W V V Q G G V L T G V W V I A H E C G H H A F S D Y Q W V D D T V G F V helianfad2.PRO
W P I H G F V Q G C F L T G H W V I A H E C G H H A F S D Y Q W L D D T V G L I petroselFad2.PRO
W P I Y W I C Q G C V C T G L W V N A H E C G H H A F S D Y Q W V D D T V G L I potatoFad2.PRO
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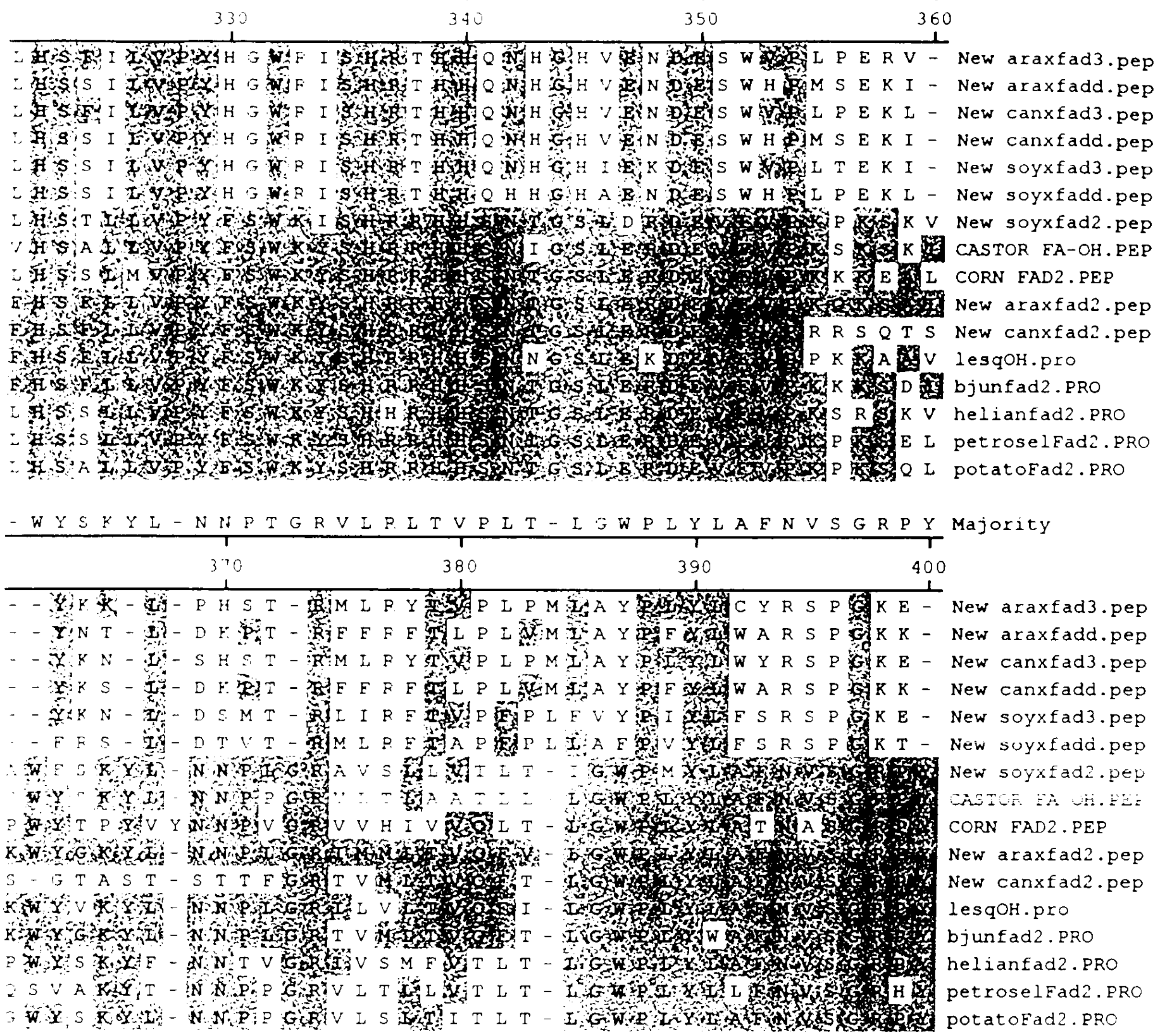

```
L H S S L L V P Y F S W K Y S H R   H H S N T G S L E R D E V F V P K P K   A -  Majority
                  330                 340                 350                 360
L H S F I L V P Y H G W F I S H R T H H Q N H G H V E N D E S W V P L P E R V -  New araxfad3.pep
L H S S I L V P Y H G W F I S H R T H H Q N H G H V E N D E S W H P M S E K I -  New araxfadd.pep
L H S F I L V P Y H G W F I S H R T H H Q N H G H V E N D E S W V P L P E K L -  New canxfad3.pep
L H S S I L V P Y H G W P I S H R T H H Q N H G H V E N D E S W H P M S E K I -  New canxfadd.pep
L H S S I L V P Y H G W P I S H R T H H Q N H G H I E K D E S W V P L T E K I -  New soyxfad3.pep
L H S S I L V P Y H G W R I S H R T H H Q H H G H A E N D E S W H P L P E K L -  New soyxfadd.pep
L H S T L L V P Y F S W K I S H R R H H S N T G S L D R D E V F V P K P K S K V  New soyxfad2.pep
V H S A L L V P Y F S W K Y S H R R H H S N I G S L E R D E V F V P K S K S K [illegible]  CASTOR FA-OH.PEP
L H S S L M V P Y F S W K Y S H R R H H S N T G S L E R D E V F V P K K K E A L  CORN FAD2.PEP
F H S K L L V P Y F S W K Y S H R R H H S N T G S L E R D E V F V P K Q K S A L  New araxfad2.pep
F H S F L L V P Y F S W K Y S H R R H H S N T G S L E R D E V F V P R R S Q T S  New canxfad2.pep
F H S E L L V P Y F S W K Y S H R R H H S N N G S L E K D E V F V P P K K A A V  lesqOH.pro
F H S F L L V P Y F S W K Y S H R R H H S N T G S L E R D E V F V P K K K S D [illegible]  bjunfad2.PRO
L H S S L L V P Y F S W K Y S H H R H H S N T G S L E R D E V F V P K S R S K V  helianfad2.PRO
L H S S L L V P Y F S W K Y S H R R H H S N H G S L E R D E V F V P K P K S E L  petroselFad2.PRO
L H S A L L V P Y F S W K Y S H R R H H S N T G S L E R D E V F V P K P K S Q L  potatoFad2.PRO

- W Y S K Y L - N N P T G R V L P L T V P L T - L G W P L Y L A F N V S G R P Y  Majority
                  370                 380                 390                 400
- - Y K K - L - P H S T - R M L P Y T V P L P M L A Y P L Y L C Y R S P G K E -  New araxfad3.pep
- - Y N T - L - D K P T - R F F F F T L P L V M L A Y P F Y L W A R S P G K K -  New araxfadd.pep
- - Y K N - L - S H S T - R M L P Y T V P L P M L A Y P L Y L W Y R S P G K E -  New canxfad3.pep
- - Y K S - L - D K P T - R F F R F T L P L V M L A Y P F Y L W A R S P G K K -  New canxfadd.pep
- - Y K N - L - D S M T - R L I R F T V P F P L F V Y P I Y L F S R S P G K E -  New soyxfad3.pep
- - F R S - L - D T V T - R M L R F T A P F P L L A F P V Y L F S R S P G K T -  New soyxfadd.pep
A W F S K Y L - N N P L G R A V S L L V T L T - I G W P M Y L A F N V S G R P Y  New soyxfad2.pep
- W Y S K Y L - N N P P G R V L T L A A T L L - L G W P L Y L A F N V S G R P Y  CASTOR FA-OH.PEP
P W Y T P Y V Y N N P V G R V V H I V V Q L T - L G W P L Y Y A T N A S G R P Y  CORN FAD2.PEP
K W Y G K Y L - N N P L G R I M N L T V Q F V - L G W P L Y L A F N V S G R P Y  New araxfad2.pep
S - G T A S T - S T T F G R T V M L T V Q F T - L G W P L Y L A F N V S G R P Y  New canxfad2.pep
K W Y V K Y L - N N P L G R I L V L T V Q S I - L G W P L Y L A F N V S G R P Y  lesqOH.pro
K W Y G K Y L - N N P L G R T V M D T V Q F T - L G W P L Y W A F N V S G R P Y  bjunfad2.PRO
P W Y S K Y F - N N T V G R I V S M F V T L T - L G W P L Y L A F N V S G R P Y  helianfad2.PRO
Q S V A K Y T - N N P P G R V L T L L V T L T - L G W P L Y L L F N V S G R H Y  petroselFad2.PRO
G W Y S K Y L - N N P P G R V L S L T I T L T - L G W P L Y L A F N V S G R P Y  potatoFad2.PRO
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D - - F A S H F D P Y S P I F N D . E R L Q I Y I S D A - - G I L A V C L . L Y Majority
                  410                       420                       430                       440
- - - - G S H F N P Y S S L F A P S E R K L Q A T S T T C W S I M F V S L I - - New araxfad3.pep
- - - - G S H Y H P D S D L F L P K E R K D V L T S T A C W T A M A A L L V - - New araxfadd.pep
- - - - G S H Y N P Y S S L F A P S E R K L I A T S T T C W S I M L A T L V - - New canxfad3.pep
- - - - G S H Y H P D S D L F L P K E R N D V L T S T A C W T A M A V L L V - - New canxfadd.pep
- - - - G S H F N P Y S N L F P P S E R K G T A I S T L C W A T M F S L L I - - New soyxfad3.pep
- - - - G S H F D P S S D L F V P N G R K D V I T S T A C W A A M L G L L V - - New soyxfadd.pep
D - S F A S H Y H P Y A P I [illegible] S N [illegible] L [illegible] V S D V - - A L F S V T Y S [illegible] New soyxfad2.pep
D - P F A C H Y D P Y G P V F S E [illegible] I A D L - - G I F A T T E V [illegible] CASTOR FA-OH.PEP
F - P F A G H F D P Y G P [illegible] A [illegible] F V S D A - - G V V A V A F [illegible] CORN FAD2.PEP
D - G F A C H F F P N A P [illegible] - - G [illegible] New araxfad2.pep
D G G F A C H F H P N A P [illegible] I S D A - - G [illegible] Y [illegible] L New canxfad2.pep
D - G E A S H F F P H A P I F K [illegible] I S D A - - G I [illegible] Y [illegible] lesqOH.pro
P E G F A C H F H P N A P [illegible] V S D A - - G I L V A V C Y [illegible] bjunfad2.PRO
D - P F A C H Y V P T S P M [illegible] E [illegible] K [illegible] Y [illegible] V M S D I - - G I V I T S F I [illegible] helianfad2.PRO
E - P F A C H Y D P K S P [illegible] S [illegible] A [illegible] L V S D A - - G V [illegible] S Y [illegible] petroselFad2.PRO
D - P F A C H Y D P Y G P [illegible] N [illegible] F I S D A - - G V L G V C Y L [illegible] potatoFad2.PRO

R L A F A K G L A W V L C V Y G V P L L I V N M F L V L I T Y L Q H - - T H P S Majority
                  450                       460                       470                       480
A L S F V F G P L A V L F V [illegible] Y I [illegible] F V M W L D A V T Y L H H H G H D E K New araxfad3.pep
C L N F T I G P I Q M L F L Y G I P Y W H N V M W L D F V T Y L H H H G H E D K New araxfadd.pep
Y L S F L V G P V T V L F V Y G V P Y I H F V M W L D A V T Y L H H H G H D D K New canxfad3.pep
C L N F V M G P M Q M L F L [illegible] V I P Y W H N V M W R D F V T Y L H H H G H E D K New canxfadd.pep
Y L S F I T S P L L V L F L Y G I P Y W H F V M W L D F V T Y L H H H G H H Q K New soyxfad3.pep
G L G F V M G P I Q L L K L Y G A [illegible] Y V I F V M W L D L V T Y L H H H G H E D K New soyxfadd.pep
R V A T L F G L V W L L C V [illegible] V N G F L V T [illegible] Y L Q H - - T H F A New soyxfad2.pep
D A T M A F G L A W V M F I [illegible] V N C F L W M I T Y L Q H - - T H P A CASTOR FA-OH.PEP
K L A A A F G V W W V V F V [illegible] A [illegible] A W [illegible] - - [illegible] CORN FAD2.PEP
R Y A A A Q G M A S M T C L Y G [illegible] N A F [illegible] - - [illegible] New araxfad2.pep
P Y A A V Q G V A S M V C F L R [illegible] G [illegible] - - [illegible] New canxfad2.pep
R Y A A S Q G L T A M L C V Y G [illegible] F [illegible] V T F [illegible] - - T H P S lesqOH.pro
R Y A A A Q G V A S M V C L [illegible] - - T H P S bjunfad2.PRO
R V A M A F G L V W V I G V [illegible] M V [illegible] - - T H P G helianfad2.PRO
R L A V A F G L T W V L C V [illegible] G [illegible] V [illegible] G F L V L I T F L Q H - - T H P A petroselFad2.PRO
R I A L V K G L A W L V C V [illegible] V [illegible] G F L V L I T Y L Q H - - T H P S potatoFad2.PRO
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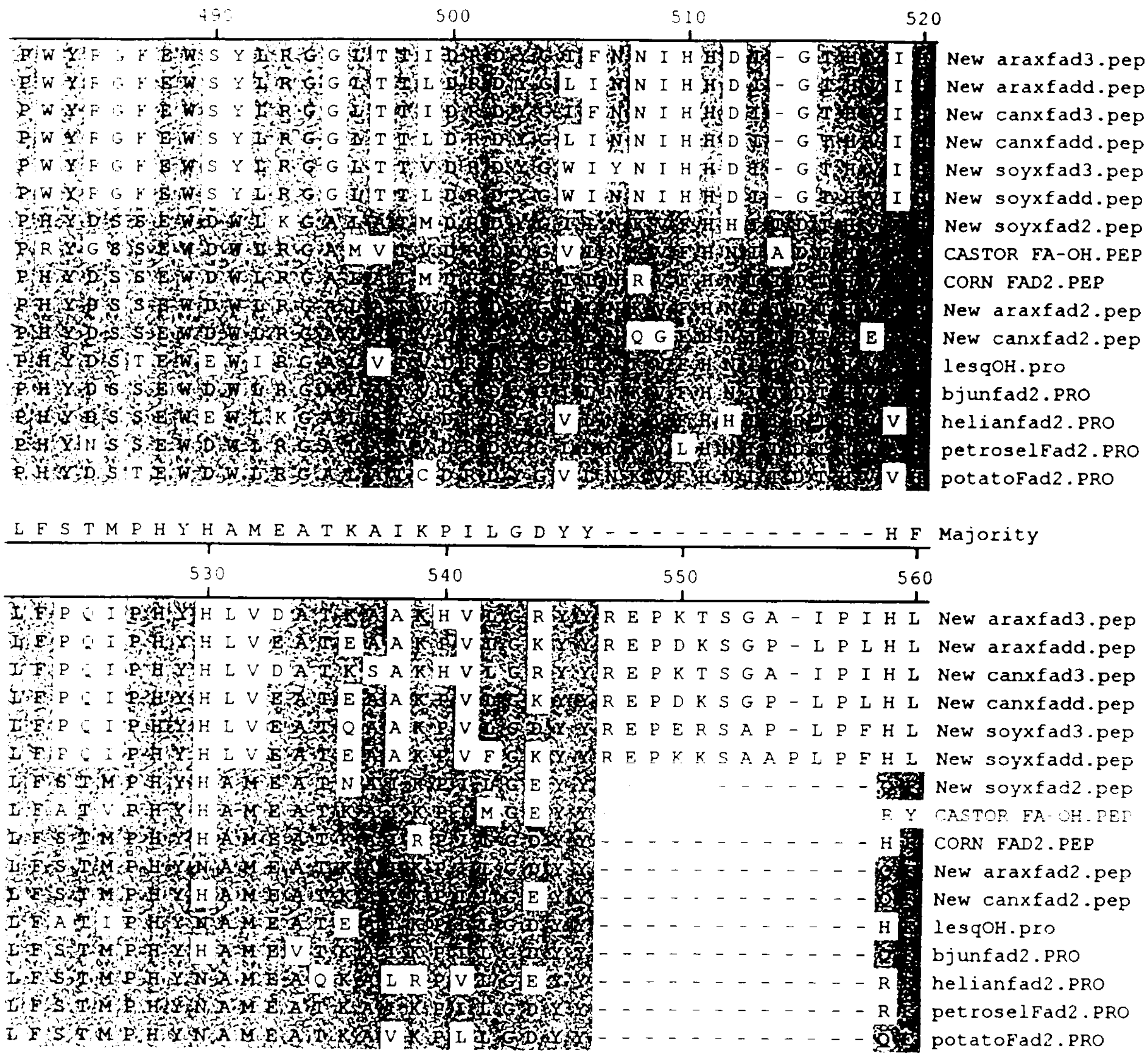
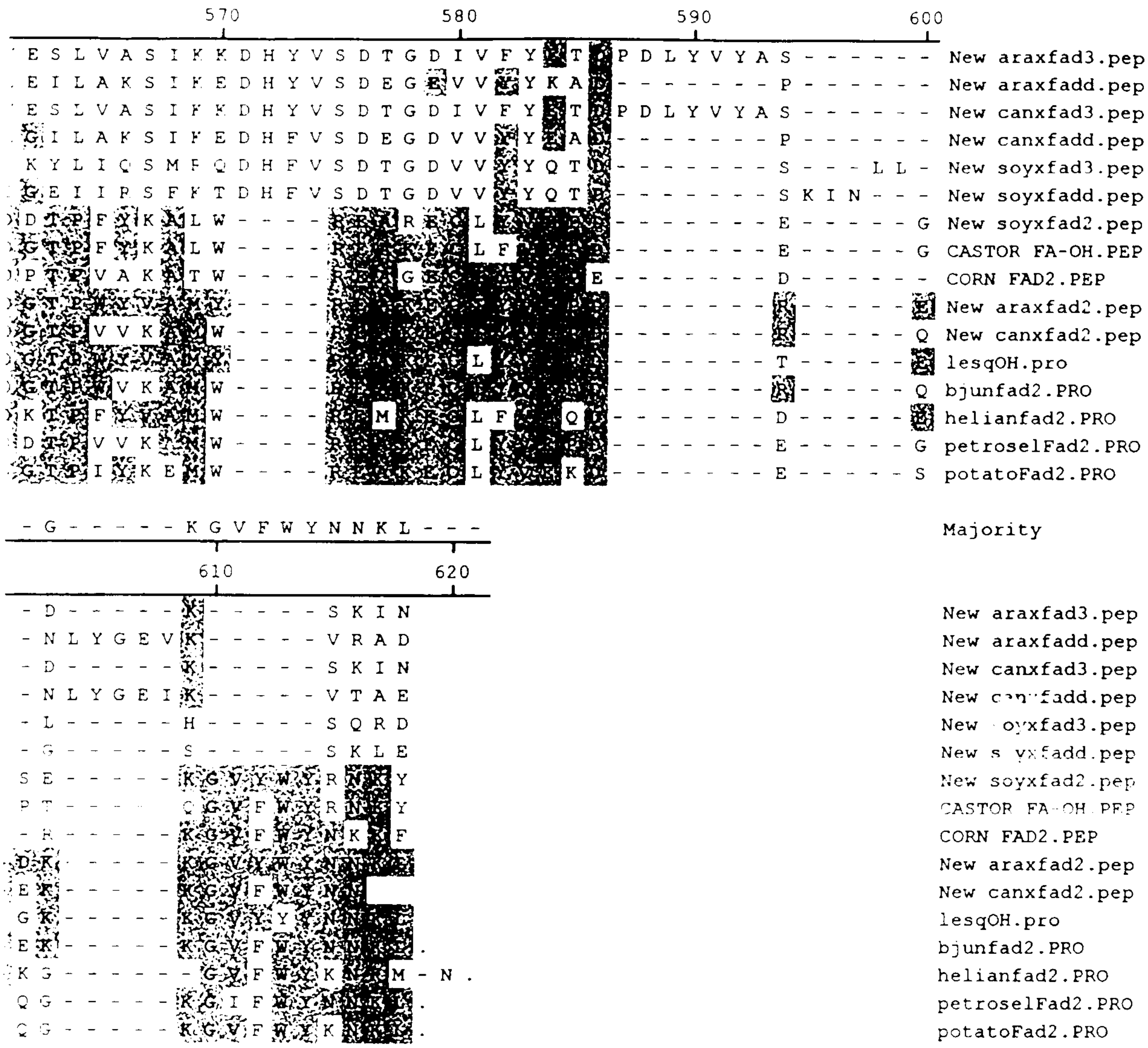

P H Y D S S E W D W L R G A L A T D R D Y G I L N K V F H N I T D T H V . H Majority
490
500
510
520
New araxfad3.pep
New araxfadd.pep
New canxfad3.pep
New canxfadd.pep
New soyxfad3.pep
New soyxfadd.pep
New soyxfad2.pep
CASTOR FA-OH.PEP
CORN FAD2.PEP
New araxfad2.pep
New canxfad2.pep
lesqOH.pro
bjunfad2.PRO
helianfad2.PRO
petroselFad2.PRO
potatoFad2.PRO
L F S T M P H Y H A M E A T K A I K P I L G D Y Y - - - - - - - - - - - - - H F Majority
530
540
550
560
New araxfad3.pep
New araxfadd.pep
New canxfad3.pep
New canxfadd.pep
New soyxfad3.pep
New soyxfadd.pep
New soyxfad2.pep
CASTOR FA-OH.PEP
CORN FAD2.PEP
New araxfad2.pep
New canxfad2.pep
lesqOH.pro
bjunfad2.PRO
helianfad2.PRO
petroselFad2.PRO
potatoFad2.PRO



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  G T P V Y K A M W - - - - R E A K E C V Y V E P D - - - - - - - S - - - - - -   Majority

                      570                      580                      590                      600

  E S L V A S I K K D H Y V S D T G D I V F Y [illegible] T [illegible] P D L Y V Y A S - - - - - -   New araxfad3.pep
  E I L A K S I K E D H Y V S D E G E V V [illegible] Y K A [illegible] - - - - - - - P - - - - - -   New araxfadd.pep
  E S L V A S I K K D H Y V S D T G D I V F Y [illegible] T [illegible] P D L Y V Y A S - - - - - -   New canxfad3.pep
  G I L A K S I K E D H F V S D E G D V V [illegible] Y [illegible] A [illegible] - - - - - - - P - - - - - -   New canxfadd.pep
  K Y L I Q S M F Q D H F V S D T G D V V [illegible] Y Q T [illegible] - - - - - - - S - - - L L -   New soyxfad3.pep
  G E I I R S F K T D H F V S D T G D V V [illegible] Y Q T [illegible] - - - - - - - S K I N - - -   New soyxfadd.pep
  D T P F Y K A L W - - - - R E [illegible] R E [illegible] L [illegible] - - - - - - - E - - - - - G   New soyxfad2.pep
  G T P F Y K A L W - - - - R [illegible] L F [illegible] - - - - - - - E - - - - - G   CASTOR FA-OH.PEP
  P T P V A K A T W - - - - R E A G E [illegible] E - - - - - - - D - - - - - -   CORN FAD2.PEP
  G T P W Y V A M Y - - - - [illegible] - - - - - - - R - - - - - E   New araxfad2.pep
  G T P V V K A M W - - - - [illegible] - - - - - - - R - - - - - Q   New canxfad2.pep
  G T P W Y V A M Y - - - - [illegible] L [illegible] - - - - - - - T - - - - - E   lesqOH.pro
  G T P W V K A M W - - - - [illegible] - - - - - - - R - - - - - Q   bjunfad2.PRO
  K T P F Y V A M W - - - - [illegible] M [illegible] L F [illegible] Q [illegible] - - - - - - - D - - - - - E   helianfad2.PRO
  D T P V V K A M W - - - - [illegible] L [illegible] - - - - - - - E - - - - - G   petroselFad2.PRO
  G T P I Y K E M W - - - - R [illegible] E D L [illegible] K [illegible] - - - - - - - E - - - - - S   potatoFad2.PRO

  - G - - - - - K G V F W Y N N K L - - -   Majority

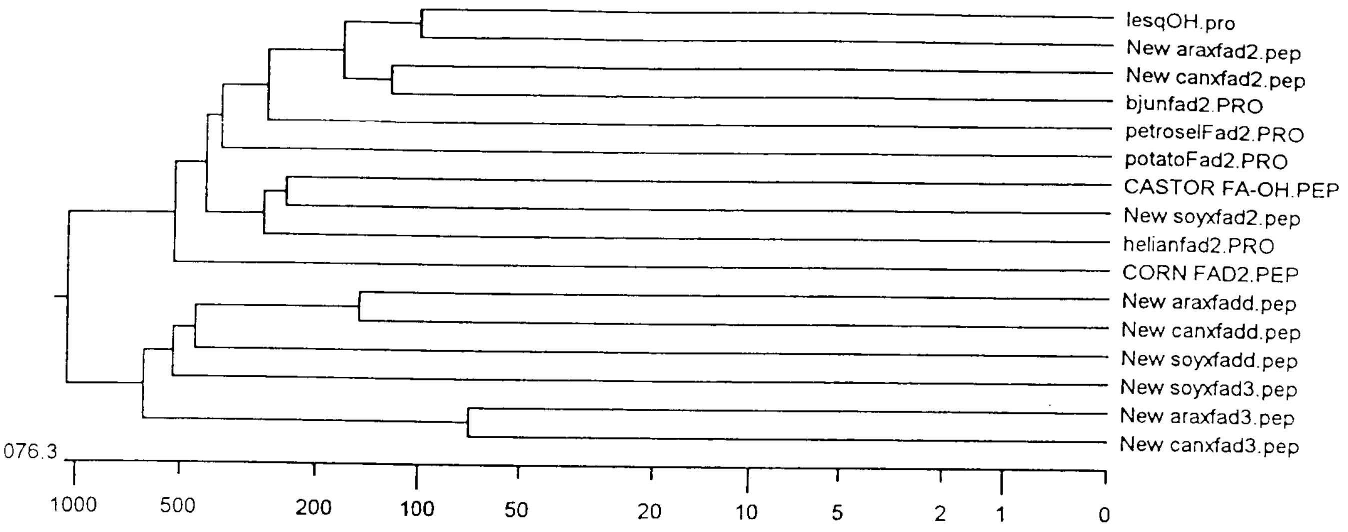
                610                      620

  - D - - - - - K - - - - - S K I N   New araxfad3.pep
  - N L Y G E V K - - - - - V R A D   New araxfadd.pep
  - D - - - - - K - - - - - S K I N   New canxfad3.pep
  - N L Y G E I K - - - - - V T A E   New canxfadd.pep
  - L - - - - - H - - - - - S Q R D   New soyxfad3.pep
  - G - - - - - S - - - - - S K L E   New soyxfadd.pep
  S E - - - - - K G V Y W Y R N K Y   New soyxfad2.pep
  P T - - - - - Q G V F W Y R N [illegible] Y   CASTOR FA-OH.PEP
  - R - - - - - K G V F W Y N K [illegible] F   CORN FAD2.PEP
  D K - - - - - [illegible]   New araxfad2.pep
  E K - - - - - K G V F W Y N [illegible]   New canxfad2.pep
  G K - - - - - K G V Y Y [illegible] N [illegible]   lesqOH.pro
  E K - - - - - K G V F W Y N [illegible] .   bjunfad2.PRO
  K G - - - - - - G V F W Y K [illegible] M - N .   helianfad2.PRO
  Q G - - - - - K G I F W Y N [illegible] .   petroselFad2.PRO
  Q G - - - - - K G V F W Y K [illegible] .   potatoFad2.PRO
```

ation 'Decoration #1': Shade (with bright turquoise at 40% fill) residues that match araxfad2.pep exactly.

Attachment VI

logenetic tree of USPTO3.MEG; using J. Hein method with PAM250 residue weight table.
nday, January 26, 1998 5:41 PM



New soyxfad2.pep = soybean delta-12 desaturase
CASTOR FA-OH.PEP = castor 12-hydroxylase
New araxfad2.pep = *Arabidopsis* delta-12 desaturase
New canxfad2.pep = canola delta-12 desaturase
CORN FAD2.PEP = corn delta-12 desaturase
bjunfad2.PRO = *Brassica juncea* delta-12 desaturase
helianfad2.PRO = sunflower delta-12 desaturase
petroselFad2.PRO = parsley delta-12 desaturase
potatoFad2.PRO = potato delta-12 desaturase
lesqOH = *Lesquerella fendleri* 12-hydroxylase
New araxfadd.pep = *Arabidopsis* plastidic delta-15 desaturase
New canxfadd.pep = canola plastidic delta-15 desaturase
New soyxfadd = soybean plastidic delta-15 desaturase
New soyxfad3 = soybean microsomal delta-15 desaturase
New araxfad3.pep = *Arabidopsis* microsomal delta-15 desaturase
New canxfad3.pep = canola microsomal delta-15 desaturase

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Edited by

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and

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EXPRESSION OF CASTOR AND *L. FENDLERI* OLEATE 12-HYDROXYLASES IN TRANSGENIC PLANTS

Effects on lipid metabolism and inferences on structure-function relationships in fatty acid hydroxylases

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Introduction

Ricinoleic acid (D-12-hydroxyoctadec-cis-9-enoic acid), is an hydroxylated fatty acid which constitutes 85-90% of the seed fatty acids in castor bean plants (*Ricinus communis L.*). This unusual fatty acid is also one of a series of related Hydroxy Fatty Acids (HFAs) produced in the seeds of *Lesquerella* species. In these species, which, like *A. thaliana* and rapeseed belong to the *Brassicacae* family, ricinoleic acid is generally a minor constituent. Major HFAs include densipolic (12-OH, 18:2 (3,9)), lesquerolic (14-OH, 20:1 (9)) and auricolic (14-OH, 20:2 (3,9)) acids.

In castor, where metabolism of HFAs has been studied in most detail, ricinoleic acid is synthesized in seeds on phosphatidyl choline, then very efficiently removed from membranes and transferred to the triacylglycerol pool (Bafor et al., 1991).

Recently, we have reported the isolation of a cDNA clone encoding the oleate 12- hydroxylase from castor (van de Loo et al, 1995). Constitutive expression of the hydroxylase cDNA in transgenic tobacco resulted in accumulation of low levels of ricinoleate in seed lipids, but not in leaves and roots. In order to further characterize metabolism of HFAs in transgenic plants, we have introduced this cDNA into *A. thaliana*. We report here how lipid metabolism is affected in transgenic plants.

Extraplastidial ω-6 desaturases and castor oleate 12- hydroxylase share a number of biochemical characteristics. Cloning of a cDNA encoding the castor hydroxylase has also confirmed that the two enzymes are closely related (van de Loo et al., 1995). Although reaction mechanisms are expected to be similar, they lead to different outcome. In order to investigate what structural components in these enzymes are [illegible] we proceeded to isolate the gene encoding another oleate 12-hydroxylase, from *Lesquerella fendleri*. Multiple comparisons of desaturase and hydroxylase sequences revealed key differences between the two categories of enzymes. We report here investigation of their functional significance.

1. Expression of Castor and *L. fendleri* Hydroxylase Genes in transgenic *A. thaliana*

We designed degenerate primers based on the sequence of castor fatty acid hydroxylase CFah12 and used them to PCR-amplify cDNAs from *L. fendleri*. One such cDNA detected an abundant seed specific transcript on Northern blots of *L. fendleri* RNA. Its sequence had extensive similarity with the CFah12 gene. This cDNA was used to isolate a genomic clone which was introduced into *A. thaliana*.

Expression of the *L. fendleri* gene resulted in accumulation of HFAs in transgenic plants, up to 15% of seed fatty acids, thus establishing the gene encodes *L. fendleri* hydroxylase LFah12.

Transgenic plants expressing CFah12 under the control of a strong seed specific promoter were also obtained. In these plants, HFAs constitute up to 20% of the seed fatty acids. Seed fatty acid composition of *A. thaliana* plants expressing CFah12 or LFah12 is very similar, suggesting the two enzymes have comparable activities in transgenic plants.

Ricinoleic acid is only one of four HFAs produced in transgenic seeds, which also accumulate densipolic, lesquerolic and a small amount of auricolic acid. This suggests that *Arabidopsis* and related *Lesquerella* species metabolize ricinoleic acid in a similar way.

Expression of LFah12 under the control of the CaMV 35S promoter did not affect fatty acid composition of vegetative organs, even though hydroxylase activity could be detected. This implies either poor enzyme activity or efficient turnover of HFAs in non-seed tissues.

Accumulation of HFAs was accompanied with an increase in oleate levels and a concurrent decrease in 18:2 and 18:3, suggesting the oleate 12- desaturase is inhibited in transgenic plants expressing either gene

2. Investigations of Structure-Function Relationships in Hydroxylases and related Desaturases

We performed multiple comparisons between oleate 12- desaturases, CFah12 and LFah12, and identified six residues conserved among desaturases which differ in fatty acid hydroxylases. Using appropriate growth conditions (Covello and Reed, 1996), we were able to express LFah12 in yeast, under the control of the *GAL1* promoter. Yeast strains over-expressing the *Lesquerella* gene accumulated a small amount of ricinoleic acid. We could also detect small levels of 18:2, indicative of some desaturase activity of the enzyme in this context.

In order to establish the functional significance of observed residue differences between desaturases and hydroxylases, we used site-directed mutagenesis to substitute desaturase residues for the corresponding residues in LFah12 at all six positions. In yeast strains expressing the mutant hydroxylase, ratios of 18:2 to ricinoleic acid levels were more than 20 fold higher than in control strains expressing the wild type gene (fig. 1). This result indicates that these residues are essential in LFah12 in determining the outcome of fatty acid oxidation

Conclusion

We described here the isolation of a novel fatty acid hydroxylase from *L. fendleri*. We also presented some results from the analysis of transgenic *A. thaliana* plants expressing the castor and *L. fendleri* genes. We plan to use these transgenic plants to dissect mechanisms involved in removing HFAs from membranes, channeling them to storage lipids or breaking them down. We also hope to gain understanding of what controls such mechanisms.

We also reported here the critical role played by a small number of residues in controlling the outcome of fatty acid oxidation. Narrowing down on fewer residues will make it easier to rationalize structural differences between hydroxylases and desaturases, and understand how these differences affect reaction mechanisms.

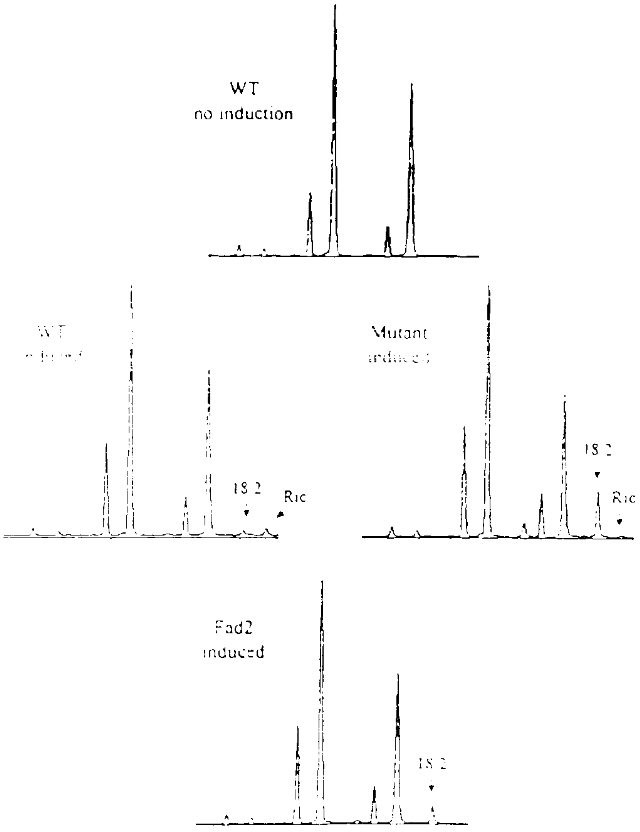

Figure 1: Gas chromatogram of fatty acid methyl esters from yeast strains expressing a wild type or a mutant fatty acid hydroxylase from *L. fendleri*

Bafor, M., Smith, M.A., Jonsson, L., Stobart, K. and Stymne, S. (1991) Ricinoleic acid biosynthesis and triacylglycerol assembly in microsomal preparations from developing castor-bean (Ricinus communis) endosperm. *Biochem. J.* 280, 507-514

Covello, P.S. and Reed, D.W. (1996) Functional expression of the extraplastidial Arabidopsis thaliana oleate desaturase gene (FAD2) in Saccharomyces cerevisiae. *Plant Physiol.* 111, 223-226

van de Loo, F.J., Broun, P., Turner, S., Somerville, C.R. (1995) An oleate 12-hydroxylase from castor (Ricinus communis L.) is a fatty acyl desaturase homologue. *Proc. Natl. Acad. Sci. USA* 92, 6743-6747

Alignment Workspace of fad2 Hein.MEG, using J. Hein method with Weighted residue weight table.

Friday, April 04, 2003 1:46 PM

```
                 ATGGGTGCT------GG------TGGTAGGATGCCGGTG--------------------GTTACTTCTT---------------------CAAGAAATG
                          10        20        30        40        50        60        70        80        9         100
D13778 (desA).SE ATGACTGCC------ACGATT[illegible]GGTTGAGGCCAACG--------------------GAAACATCTA---------------------CAA------ 48
CDS SEQ ID 1.SEQ ATGGGTGCA------GG------TGGAAGAATGCCGGTT--------------------CCTACTTCTT---------------------CAAGAAATC 47
CDS SEQ ID 3.SEQ ATGG[illegible]CA------GG------TGGAAGAATGCAAGTG--------------------TCT[illegible]TCGCT---------------------CAAAAAGTC 47
CDS SEQ ID 5.SEQ ATGGGAGGT------AG------AGGTCGTGTGGCCAAA--------------------GT-----------------------------GAACTTCAA 39
CDS SEQ ID 7.SEQ ATGGGT[illegible]CAGGATGACCGAGAAGGAGCGGGAGAAG[illegible]A[illegible]GAGTANGMTTHN---------------------TY[illegible]GTARGG 80
CDS SEQ ID 11.SE ATGGGAGGT------GG------TGGTCGCATGTCTACT--------------------GT---------CATAATCAGCAA[illegible]GT AGAAGAAAGG 59

                 TGGAAGCGGCAA-----CACCGAGCGCGTGCCGATCGCGAAGC[illegible] TCACTG---------------------TTGGAGA[illegible]ATC[illegible]CACCGC
                          110       120       130       140       150       160       170       180       190       200
D13778 (desA).SE ---------------------------TCCCGATCGCCCGATT[illegible]TAAAA---------------------TACAAGA[illegible]TG[illegible]CCAAGG 106
CDS SEQ ID 1.SEQ GGAAACCGACAC-----CACAAAGCGTGTGCCGTGCGAGAAAC[illegible]TCTCGA---------------------[illegible] 127
CDS SEQ ID 3.SEQ TGAAACCGACAA-----CATCAAGCGCGTACCCTGCGAGACAC[illegible]TCACTG---------------------[illegible]CACTGC 127
CDS SEQ ID 5.SEQ GGCAAGAAGCCT-----CTCTCAA-GGATCCCAAACACAAAGCCACCG TCACTG---------------------TTGGCCAAGTCAAG[illegible]TT CACCAC 114
CDS SEQ ID 7.SEQ [illegible] 180
CDS SEQ ID 11.SE ACGAAGCAGCCA-----CCGGAGCGAGCGCCGCACACGAAGC[illegible] A[illegible]A[illegible]A[illegible]---------------------[illegible]CACCGC 139

                 ACTGCTTCGAGCGCTCGGTCCTCCGCTCTTTCTCCTACGTTGT[illegible]TAT[illegible]CTTCATTATAGCCT[illegible]TTTT[illegible]TACTA[illegible]CCTCT
                          210       220       230       240       250       260       270       280       290       300
D13778 (desA).SE AATGCTTCGAGAAAAAAGC[illegible]AGCAAAGCT---------GGGCTTC[illegible]TTTGATTACC[illegible]TAGGGGGAT[illegible]GCCTTGGGCTA[illegible]GCA[illegible]TATTT---- 194
CDS SEQ ID 1.SEQ ATTGTTTCAAACGCT[illegible]AATCCCTCGCTCTTTCTCCTACCTTAT[illegible]CATCATTATAG[illegible]TA[illegible]TA[illegible]A[illegible]CTTCTCTCT 227
CDS SEQ ID 3.SEQ ACTGTTTCAAACGC[illegible]CGATCCCTCGCT[illegible]TTTCTCCTACCTCAT[illegible]----CATCATCATAGCC[illegible]TA[illegible]TA[illegible]A[illegible]CCTCT 227
CDS SEQ ID 5.SEQ ACTGCTTTCAGCGC[illegible]CCT[illegible]TCA[illegible]TCATTCTCCTATGTTGTTTAT[illegible]CATTTGC[illegible]ATTT[illegible]TAC---ATTGC[illegible]A[illegible]CTTCCACCT 215
CDS SEQ ID 7.SEQ ACTGCTTCGAGCGCTCGGTGCTCAAGTCCTTCTCGTACGTGGTC[illegible]A[illegible]TGGTGAT[illegible]CTCTACTTCGC[illegible]CATACCCGC 280
CDS SEQ ID 11.SE ATTGCTTTGAACGCTCTTTTGTGCGCTCATCTCCAATTTGC[illegible]TATA[illegible]TTTCTGCTTAA[illegible]TTTC[illegible]TA[illegible]A[illegible]TTCCCTA 234

                 CCTCCCTCACCCGCTCTCCTACTTCGCTGGCCTCTCTACTGG[illegible]CAAGGT[illegible]GCGT[illegible]ACTGG[illegible]GT[illegible]CAT[illegible]TGTGGCCAC
                          310       320       330       340       350       360       370       380       390       400
D13778 (desA).SE --------A[illegible]TT[illegible]ACT---TTTTGCTTTTACCTGGAT[illegible]ACA[illegible]AACA[illegible]TTAA[illegible]TGT[illegible]CCAC 282
CDS SEQ ID 1.SEQ CCTCCCTCAGCCTCTCTCTTACTTGGCTTGGCCACTCTATTGG[illegible]CAAGG[illegible]TGT[illegible]CTAACTGGTAT[illegible]CAT[illegible]TGTGGCTAC 327
CDS SEQ ID 3.SEQ CCTC[illegible]CACTTC[illegible]TACTT[illegible]CTTGGCCTCTCTACTGG[illegible]CAGGA[illegible]CTAA[illegible]CAT[illegible]TG[illegible]CAC 32[illegible]
CDS SEQ ID 5.SEQ CCTTCCTCAACCCTTTTCC TCATTGCATGGCCAATCTATTGG[illegible]CAAGGTTGCCTTCT[illegible]ACTGGT[illegible]GAT[illegible]TGTGGTCAC 315
CDS SEQ ID 7.SEQ GCTCC[illegible]AAGCCCGCTCGGCTACGCCGCCTGGCCGCTGTACTGGATC[illegible]CAGGGG[illegible]CAC[illegible]TGT[illegible]CAT[illegible]TGTGGCCAC 380
CDS SEQ ID 11.SE CATCTCTTCTCCGCTCTCGTATGTCGCTTGGCTGGTTTACTGGCTCTT CAAGG[illegible]CATT[illegible]TTTGGCTCATC[illegible]TGTGGCCAT 334
```

Alignment Workspace of fad2 Hein.MEG, using J. Hein method with Weighted residue weight table.
Friday, April 04, 2003 1:46 PM

```
                  CAGG[illegible]TTCAGGGA[illegible]TA[illegible]CAGT[illegible]GATGACATT[illegible]TGG[illegible]A[illegible]TTACT[illegible]AT[illegible]C
                       410       420       430       440       450       460       470       480       490       500

D13778 (desA).SE  [illegible]TTT[illegible]AAAAAAA[illegible]TT[illegible]AATGATTTAGT[illegible]GATTTA[illegible]TAT[illegible]TCCAGGACC 3[illegible]
CDS SEQ ID 1.SEQ  CAGG[illegible]TTCAGGGACTACCAATG[illegible]GGATGACACATTGG[illegible]TTA[illegible]TTTA[illegible]GCTATCCC 4[illegible]
CDS SEQ ID 3.SEQ  CA[illegible]TT[illegible]A[illegible]TA[illegible]AGT[illegible]GACGACA[illegible]ATCGAC 42[illegible]
CDS SEQ ID 5.SEQ  CATG[illegible]TCAGCAAGTACCAAT[illegible]GATGATGTTGTGGGTTTGA[illegible]TT[illegible]AACTATTTTTA[illegible]TTTATTTCTA[illegible]ATCCC 41[illegible]
CDS SEQ ID 7.SEQ  CA[illegible]TTGGACTACT[illegible]GACGACTGGT[illegible]TTATT[illegible]ACCCC 4[illegible]
CDS SEQ ID 11.SE  CATGCTTTTAG[illegible]AGTATCAG[illegible]GATGACATT[illegible]TTG[illegible]AA[illegible]TATCCC 43[illegible]

                  GCCACCATT[illegible]CAA[illegible]ACT[illegible]GGTTCC[illegible]TGGAGCGAGACGAAGTGTTTGT[illegible]GAAGAAGAAGTGG[illegible]AATT[illegible]TCGTA[illegible]CAACAACCC
                       510       520       530       540       550       560       570       580       590       600

D13778 (desA).SE  ACCACCATCTCCACACCAATAAAATT[illegible]AAGTGGACAA[illegible]TTTGGA[illegible]AGA[illegible]TTTTGA--AGCTA[illegible]TT[illegible]TATTT 4[illegible]
CDS SEQ ID 1.SEQ  GTCACCATT[illegible]CAA[illegible]ATGGATCC[illegible]TGAAAGAGATGAAGTATTTGT[illegible]AAAG[illegible]AAAT[illegible]AATCAA[illegible]TTA[illegible]CAACAACCC 524
CDS SEQ ID 3.SEQ  [illegible]TCAACATTGGTTCC[illegible]AGAGAGACGAAGTGTTT[illegible]GAAGAAGAA[illegible]AGATCAA[illegible]TTA[illegible]-CAACAACCC 524
CDS SEQ ID 5.SEQ  G[illegible]ATCACTCCAACACAGGTTCC[illegible]TTGACGGTGATGAAGTGTTT[illegible]AAAACCAAAATC[illegible]AAA[illegible]TTTAT[illegible]AACAACCC 51[illegible]
CDS SEQ ID 7.SEQ  GCCA[illegible]CAACA[illegible]GGAGCGAGACGAAGTGTTT[illegible]GAAGAAGAA[illegible]TTA[illegible]CAACAACCC 58[illegible]
CDS SEQ ID 11.SE  [illegible]CA[illegible]TAACATAGGATCT[illegible]TTGAGCGAGACGAAGTGTT[illegible]GAAATCAAAC[illegible]AAAATTTCATCCTATTCTA[illegible]AAACAACCC 536

                  TCTC[illegible]-GACGGGTTGTGACGCTTGTGG[illegible]CAGCTGACCCTGGGT[illegible]TTTTGTA[illegible]TTAGC[illegible]CAATG[illegible]TCG[illegible]GTAGA[illegible]TAT[illegible]AT[illegible]---CTTTGC
                       610       620       630       640       650       660       670       680       690       700

D13778 (desA).SE  TA[illegible]ATG[illegible]TTCT[illegible]A---------[illegible]ATTT[illegible]CAGTGGGTTTAATG------------[illegible]------------ 5[illegible]
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CDS SEQ ID 3.SEQ  TTTG[illegible]-GA[illegible]CA[illegible]CATCTTAAC[illegible]TTCAGTTCAGTCT[illegible]TTTGTACTTA[illegible]AA[illegible]GCAGAC[illegible]TA[illegible]GCCCTTTGC 62[illegible]
CDS SEQ ID 5.SEQ  [illegible]TAG-GAA[illegible]TGTTTC[illegible]TTCT[illegible]ACCACAATAG[illegible]TATGTATTTAG[illegible]AAT[illegible]GTAGA[illegible]TAT[illegible]ATA---TTTTGC 60[illegible]
CDS SEQ ID 7.SEQ  GTTT[illegible]-[illegible]CCAGATT[illegible]CAG[illegible]TTGGA[illegible]GA[illegible]TAA[illegible]---CTTTGC 67[illegible]
CDS SEQ ID 11.SE  GCCAG-[illegible]TCGAGTTTTGACACTT[illegible]CA[illegible]TTTATATTTAGCTT[illegible]AAC[illegible]GTAGA[illegible]TAT[illegible]AT[illegible]---CTTTGC 63[illegible]

                  TTGCCATTT[illegible]GA[illegible]TAC[illegible]CTCC[illegible]AT[illegible]TACAAGGACCGTGAG[illegible]GAGATCTACATT[illegible]AC[illegible]TTGGATTCTT[illegible]TTTAC
                       710       720       730       740       750       760       770       780       790       800

D13778 (desA).SE  -----ACTTTAAACT[illegible]CCAACTTTG[illegible]CCA[illegible]GGAAC[illegible]CAATAAA[illegible]AAACT[illegible]CATT---[illegible]TCTTTCTCTT[illegible]CCTTT[illegible]CT 63[illegible]
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CDS SEQ ID 3.SEQ  TTGCCATTT[illegible]CA[illegible]AA[illegible]AT[illegible]TACAAGGA[illegible]CAGATATACATAT[illegible]TCTCTAC 72[illegible]
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CDS SEQ ID 7.SEQ  CTCCCATTT[illegible]GA[illegible]TAC[illegible]AT[illegible]TACAAGGA[illegible]CAGAT[illegible]TCTCTAC 77[illegible]
CDS SEQ ID 11.SE  TTGCCATTATGATCCCTATGGCCAATATTTTCCGAAAGAGAAACGTT[illegible]CAGATTTACATTGCTGACCT[illegible]GAATCTTGCCACAA[illegible]TTTTAT [illegible]
```

Alignment Workspace of fad2 Hein.MEG, using J. Hein method with Weighted residue weight table.

Friday, April 04, 2003 1:46 PM

```
                    GGTTTTGCTGCTGCAAAGGGGTGGTTTGGGTGCTCTGCTTGTA[illegible]
                         810       820       830       840       850       860       870       880       890       900

D13778 (desA).SE    GCTTTGATCATCATTAC[illegible]GAGTGT[illegible]TTTTGTCAAGTTTT[illegible]                              73[illegible]
CDS SEQ ID 1.SEQ    CGTTA[illegible]T[illegible]CAAGG[illegible]GAT[illegible]GAT[illegible]GAT[illegible]GAT[illegible]T[illegible]TA[illegible]    AC[illegible]TAGTTGCAGC 80[illegible]
CDS SEQ ID 3.SEQ    [illegible]GTTA[illegible]GAT[illegible]T[illegible]TA[illegible]    ACT[illegible]TGCAGC 82[illegible]
CDS SEQ ID 5.SEQ    CGTGTT[illegible]AACC[illegible]GAAA[illegible]GGTT[illegible]TGCTATGTGTTTAT[illegible]    ATTTGCAGC 80[illegible]
CDS SEQ ID 7.SEQ    AAG[illegible]G[illegible]GTG[illegible]GTTA[illegible]GCTGATT[illegible]GAA[illegible]    ACTTGCAGC 80[illegible]
CDS SEQ ID 11.SE    CAGGCTACAATGGCAAAAGGGTTGGTTGGGTAATG[illegible]TAT[illegible]TAT[illegible]GCTTATTGTTAACTGTTTG[illegible]    ACTTGCAGC 8[illegible]

                    ACACTCATCCTTCGTTGCCTCACTATGACTCATCAGAGTGGGA[illegible]GAGGGGAGCTTTGGGT---A[illegible]GGGACAGAG[illegible]TA[illegible]GTTGAACAA
                         910       920       930       940       950       960       970       980       990      1000

D13778 (desA).SE    ACACCATTCCTGAAATTGGTTTG[illegible]GTGAAGATTGGAGT[illegible]GAA[illegible]CAGTTAAATGGGA[illegible]TGA[illegible]GCTTGGTGA 9[illegible]
CDS SEQ ID 1.SEQ    ACACTCATCC[illegible]TGGTTGCCTCACTA[illegible]GATTCATCAGAGTGGGA[illegible]GAGGGGAGCTTTGG[illegible]---A[illegible]GTACAGAGA[illegible]TTGAACAA 917
CDS SEQ ID 3.SEQ    ACACGCAT[illegible]TTCGTTGCCTCACTATGACTCGTCTGAGTGGGAT[illegible]GAG[illegible]GAGCTT[illegible]---A[illegible]GTGGAGAGA[illegible]TTGAACAA 920
CDS SEQ ID 5.SEQ    ACACACA[illegible]TTTGGCTTGCTCATTA[illegible]GATTCATCAGAATGGGA[illegible]GAAGGGAGCTTTGGA---ACTATGGACAGAG[illegible]TCTGAACAA 905
CDS SEQ ID 7.SEQ    ACACCCA[illegible]GGTGGTTGCCCACTACGACTCGACGGACTGGGA[illegible]GGGGAGCTTGG[illegible]---A[illegible]ATGGAC[illegible]TTGAACG 91[illegible]
CDS SEQ ID 11.SE    ACACTCACCCAGCTATTCTACGTTATGGCTCATCGGAATGGCATT[illegible]GGGAGTAATGG[illegible]---ACTGTGGATAGAG[illegible]TTGAATAA 92[illegible]

                    GGTGTTGCACAATATCACTGACATGCATGTGGCTCATCACCTCTTCT[illegible]ATCAT[illegible]GGCATTACCATGGAATGAA[illegible]A[illegible]GAAGCCCATA
                         1010      1020      1030      1040      1050      1060      1070      1080      1090      1100

D13778 (desA).SE    AGTACTTGGCACGACATTAATGTGCATATT[illegible]CATCAC[illegible]GCATT[illegible]CTAGAATTT[illegible]GTTTAC[illegible]--[illegible]TGAAAGAGA 931
CDS SEQ ID 1.SEQ    GGTGTT[illegible]ACAATATTAGAGACAGACGGTGGTCATCACCTCTTT[illegible]ACAAT[illegible]GGCATTACAA[illegible]TGG[illegible]A[illegible]AAGCCAATT 101[illegible]
CDS SEQ ID 3.SEQ    GGTGTT[illegible]ACAATATCACGGACACGCACGTGGCGCATCACCTGTTC[illegible]ACCAT[illegible]GGCATTACCATGGAATG[illegible]A[illegible]AAGCCCATA 102[illegible]
CDS SEQ ID 5.SEQ    GGTGTTTATTACATAAATGATACTTATGTGGCTCACCATCTCTT[illegible]ACAAT[illegible]CACATTACATGGAATGAAT[illegible]A[illegible]AAGCCAATA 100[illegible]
CDS SEQ ID 7.SEQ    GGTGTTCACAACATTACCGACACGCACCTGCTGCACCACCTCTTC[illegible]ACCAT[illegible]GGCATTACGA[illegible]TGG[illegible]A[illegible]AAGCCCCTC 107[illegible]
CDS SEQ ID 11.SE    AGTATTTCATAACATTGTAGACAC[illegible]CATGTAGCTCATCATCTTTTT[illegible]ACAATT[illegible]CCAGATTA[illegible]CAT[illegible]GAATGAA[illegible]A[illegible]AAGCCTTA 102[illegible]

                    CTGGGTCAGTATTACCAGTTTGATGGGACCCGGTTTTACAAGGCGAT[illegible]GAGGGAGGGAGGCAGGTAGT[illegible]TATTC[illegible]TA[illegible]AGCTGCA
                         1110      1120      1130      1140      1150      1160      1170      1180      1190      1200

D13778 (desA).SE    AGTGGGTATC-TTTTTTGTAGGAACGTACTTTCAACTGGGGG[illegible]A[illegible]AAGAATGTA[illegible]A[illegible]TATTTGTACGATG[illegible]TGA[illegible]-------ATG 1021
CDS SEQ ID 1.SEQ    CTGGGACAGTATTACCAGTTTGATGGAACACCGTGGTATGTAGCGAT[illegible]ATAGGGAGGGAAAGGAGTTGTATCTATTCTAGAC[illegible]TGA[illegible]AAGCTGACA 111[illegible]
CDS SEQ ID 3.SEQ    CTGGGACAGTATTATCAGTTTGATGGGACGCCGGTGGTTAAGG[illegible]GAT[illegible]GAGGGAGGGAGCGAAGGAGTGTAGTATTC[illegible]GAAC[illegible]TGA[illegible]AGCTGCA 112[illegible]
CDS SEQ ID 5.SEQ    TTGGGTCAGTACTATCAATTTGATGACACACCATTTTACAAGG[illegible]AT[illegible]GGAGAGAAGGAGAGAGTAGT[illegible]CTATT[illegible]AA[illegible]TGA[illegible]AACATCG 110[illegible]
CDS SEQ ID 7.SEQ    [illegible]GG[illegible]CAGTACTACCAGTTCGACGGCACG[illegible]TGTTCCAAGG[illegible]GAT[illegible]GAGGGAGCGGAG[illegible]AAGTAGTGTATGGTGAGC[illegible]TGA[illegible]-- ------ATG 1164
CDS SEQ ID 11.SE    ATGGGTCAGTATTACGGGTATGATGGAA[illegible]TATTTACAAGG[illegible]GAT[illegible]GGAGGGAGGGAAAGAGTAGTATTCTTTGTAGAC[illegible]TGA[illegible]AGCTGCTA 112[illegible]
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                   AGAAAGGCGTGTTCTGGTACAACAATAAGTTTTGA
                             1210      1220      1230

D13778 (desA).SE GCTACCGCACCTTTAGCTCCCTCAAA------TAA     10[illegible]
CDS SEQ ID 1.SEQ AGAAAGGTGTGTACTGGTACAACAATAAGTTATGA     11[illegible]
CDS SEQ ID 3.SEQ AGAAAGGTGTGTTCTGGTACAACAATAAGTTATGA     11[illegible]
CDS SEQ ID 5.SEQ AGAAGGGCGTGTATTGGTACAGGAACAAGTATTGA     11[illegible]
CDS SEQ ID 7.SEQ GCAAGGGCGTCTTCTGGTACAACAAGAAGTTCTAG     11[illegible]
CDS SEQ ID 11.SE CACAAGGCGTTTTCTGGTACCGGAACAAGTATTAA     1164
```

Sequence pair distances of fad2 Hein.MEG, using J. Hein method with Weighted residue weight table.
Friday, April 04, 2003 1:46 PM

Percent Identity

Divergence

	1	2	3	4	5	6	
1	■	39.6	40.8	38.8	45.8	39.8	**1**
2	100.0	■	85.7	66.0	63.5	65.3	**2**
3	100.0	16.0	■	65.8	67.5	64.8	**3**
4	100.0	46.1	46.6	■	59.9	70.6	**4**
5	97.8	50.4	42.7	57.8	■	58.9	**5**
6	100.0	47.6	48.6	38.4	60.3	■	**6**
	1	**2**	**3**	**4**	**5**	**6**	

D13778 (desA).SEQ
CDS SEQ ID 1.SEQ
CDS SEQ ID 3.SEQ
CDS SEQ ID 5.SEQ
CDS SEQ ID 7.SEQ
CDS SEQ ID 11.SEQ

Alignment Workspace of 1043-D suppl.:MEG, using Clustal method with PAM250 residue weight table.
Friday, April 04, 2003 1:43 PM

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              MGAGGRMXXVEKSPPSE-----GTPNLKFVPCEKPPFTVGDIKKAIP[illegible]CFERSILRSFSYVVYDLIIASCFYYIATTYFPL[illegible]PSP[illegible]NYIAW---PLYWA
                        10        20        30        40        50        60        70        80        9         100

desA.PRO      MTAT----------IPPLTPTVTPSNPDRPIAD---LPIQ[illegible]CFERPASKAWASVLITIGA------IA[illegible]YIGI[illegible]---[illegible]WYCIPMIWI 76
SEQ ID 2.PRO  MGAPPRM[illegible]FTSPKK[illegible]E------T[illegible]TKPVPCEKPPF[illegible]CFERSIPRSFSYLI[illegible]IIASCFYYVATNYFSL[illegible]P[illegible]W---PLYWA 91
SEQ ID 4.PRO  MTAGGRMQ[illegible]SPP[illegible]PKSE------TPNIKPVPCETPPFTVGELKKAIP[illegible]CFKRSIPRSFSHLIWDIIIASCFYYVATTYFPL[illegible]PNE[illegible]NYIAW---PLYWA 91
SEQ ID 6.PRO  MGGRGP[illegible]APVETQ---------SPKPLSFVENTKPPFTVGQIKKAIP[illegible]CFQRSLLTSFSYV[illegible]YPLSFAPIFY-IATTYFHL[illegible]P[illegible]W---PLYWI 87
SEQ ID 8.PRO  MGAGGRMTEKEREEKQEQLARATGGAAMQRSPVEKPPFTLGQIKKAIP[illegible]CFERSVLKSFSYV[illegible]HDLVIAAALIYFALAIIPA[illegible]PS[illegible]W---PLYWI 97
SEQ ID 12.PRO M[illegible]TVIIKNKPE--KPSGGGHLEPAPHTKPPFTIGDIKKAIP[illegible]CFKRSVFRSFSLFAYNF[illegible]CFLSYCIATNFFP[illegible]SP[illegible]W---LYYWL 95

              C[illegible]GCVLTGVWVIAHECGHHAFSDYQWLDDTVGLILHSFLLVPYFSWKYSHRRHHSNTGSLERDEVFVPK-KSAISW-YSKY[illegible]NN[illegible]GR[illegible]MLAVQITLG
                       110       120       130       140       150       160       170       180       190       200

desA.PRO      WTGIAL[illegible]GAFVVGHDCGHRSFAKKRWVNDLVGHIAFAPLIYPFFSWRI[illegible]HDHHHLHTNKIPVDNAWDP-------WLVEAFQ[illegible]SPI[illegible]YRAIRCPFW 16
SEQ ID 2.PRO  C[illegible]GCVLTGVWVIAHECGHHAFSDYQWLDDTVGLIFHSFLLVPYFSWKYSHRRHHSNTGSLERDEVFVPKQKSAISW-YGKY[illegible]NN[illegible]GR[illegible]MLTVQFTLG 18
SEQ ID 4.PRO  C[illegible]GCVLTGVWVIAHECGHAAFSDYQWLDDTVGLIFHSFLLVPYFSWKYSHRRHHSNTGSLERDEVFVPR-RSQISS-GTAS[illegible]-S[illegible]GR[illegible]MLTMQFTLG 18
SEQ ID 6.PRO  DQGCVLTGVWVIAHECGHHAFSKYQWVDDVVGLTLHSTLLVPYFSWKISHRRHHSNTGSLDRDEVFVPKPKSKVAW-FSKY[illegible]NN[illegible]GR[illegible]VSLVVTITIG 18
SEQ ID 8.PRO  AIGC[illegible]TGVWVIAHECGHHAFSDYS[illegible]DDVVGLVLHSSLMVPYFSWKY[illegible]HRRHHSNTGSLERDEVFVPKKKFAIPW-YTKY[illegible]NN[illegible]GR[illegible]HIVVQITLG 19
SEQ ID 12.PRO FIG[illegible]GLWVIGHECGHHAFSEYQLADDIVGLIVHSALLVPYFSWKY[illegible]HRRHHSNIGSLERDEVFVPKTKSKIGW-YSKY[illegible]NN[illegible]GR[illegible]TITLTILLG 19

              WPLYLAFNVSGRPYD[illegible]-FACHFDPYAPIYNDRERLQIYVSDAGIF---[illegible]VAFGLYRYAAA[illegible]GIAW[illegible]VIYGIP[illegible]LIVNGFL[illegible]QH[illegible]HPS[illegible]PHYDSS
                       210       220       230       240       250       260       270       280       290       300

desA.PRO      W--------TGSIFHWSLM-HFK--LSNFAQRERNKVKLSIAVVFLFAAIAFPALIITT--GVWGFVKFWLMPWLVYHFWMS[illegible]HH[illegible]IPF[illegible]PFLPAA 25
SEQ ID 2.PRO  WPLYLAFNVSGRPYDG-FACHFFPNAPIYNDRERLQIYLSDAGI[illegible]---AVCFGLYRYAAAQGMASM[illegible]YGVPLL[illegible]VNAFL[illegible]QH[illegible]HPS[illegible]PHYDSS 28
SEQ ID 4.PRO  WPLYIAFNVSGRPYDRGFACHFHPNAPIYNDRERIQIYISDAGI[illegible]---VVCYGLYRYAA[illegible]GVASM[illegible]YGVPLLIVNGFL[illegible]QH[illegible]HPS[illegible]PHYDSS 28
SEQ ID 6.PRO  WPNYLAFNVSGRPYDS-FASHYHPYAPIYSNRERLLIYVSDVALF---SVTYSLYRVATLKGLVWLLCVYGVPLLIVNGFL[illegible]QH[illegible]HFAL[illegible]PHYDSS 28
SEQ ID 8.PRO  WPLYLATNASGRPYPR-FACHFDPYGPIYNDRERAQIFVSDA[illegible]---VVAFGLYRLAAAF[illegible]YGVPLLIVNAWL[illegible]QH[illegible]HPS[illegible]PHYDSS 29
SEQ ID 12.PRO WPLYIAFNVSGRPYDR-FACHYDPYGPIFSERERLQIYIADLGIF---ATTFVLYQATMAKGLAWVMRIYGVPLLIVNCFL[illegible]QH[illegible]HPA[illegible]PHYDSS 28

              EWDWLRGAL-ATVDRDYGILNKVFHNITDTHVAHHLFSTMPHYHAME[illegible]TKAIKPILGEYYQFDGTPVYKAIWREAKECIYV[illegible]PDR[illegible]VFWYNNKL
                        310       320       330       340       350       360       370       380       390

desA.PRO      EWSAAFAQLNGIVH[illegible]YPEPW[illegible]EIL[illegible]HGINVHTPHHLSVAIPCYNLRIAH[illegible]HENWGPFI-YERTFNWQ[illegible]MQGI[illegible]HY[illegible]PRH[illegible]ISI---MKV 35
SEQ ID 2.PRO  EWDWLRGAL-ATVDRDYGILNKVFHNITDTHVAHHLFSTMPHYNAMEATKAIKPILGEYYQFDGTPWYVAMYREAKECIYV[illegible]PDR[illegible]VWYWNNKL 38
SEQ ID 4.PRO  EWDWLRGAL-ATVDRDYGILNQVFHNITDTHEAHHLFSTMPHYHAMEATKAIKPILGEYYQFDGTPVVKAMWREAKECIYV[illegible]PDR[illegible]VFWYNNKL 38
SEQ ID 6.PRO  EWDWLRGAL-ATMDRDYGILNKVFHHITDTHVAHHLFSTMPHYHAMEATNAIKPILGEYYQFDDTPFYKAIWREAKECLYV[illegible]PDR[illegible]VYWYRNKY 37
SEQ ID 8.PRO  EWDWLRGAL-AIMDRDYGILNRVFHNITDTHVAHHLFSTMPHYHAMEATKAIRPILGDYYHFDFTPVAKATWREATECIYV[illegible]P[illegible]VFWYNNKF 38
SEQ ID 12.PRO EWDWLRGAM-VTVDRDYGVLNKVFHNIADTHVAHHLFATVPHYHAMEATKAIKPIMGEYYRYDGTPFYKALWREAKECLFV[illegible]PDR[illegible]VFWYRNKY 38
```

Sequence pair distances of 1043-D suppl..MEG, using Clustal method with PAM250 residue weight table.
Friday, April 04, 2003 1:44 PM

Percent Identity

Divergence

	1	2	3	4	5	6		
1		18.5	17.9	17.1	19.7	18.2	**1**	desA.PRO
2	197.7		82.8	68.1	67.9	65.3	**2**	SEQ ID 2.PRO
3	195.0	17.3		63.6	64.8	60.1	**3**	SEQ ID 4.PRO
4	203.0	39.0	43.6		62.5	64.9	**4**	SEQ ID 6.PRO
5	184.8	37.0	40.2	45.2		58.9	**5**	SEQ ID 8.PRO
6	200.0	45.0	51.5	42.9	53.1		**6**	SED ID 12.PRO
	1	**2**	**3**	**4**	**5**	**6**		

Phylogenetic tree of 1043-D suppl..MEG, using Clustal method with PAM250 residue weight table.
Friday, April 04, 2003 1:44 PM